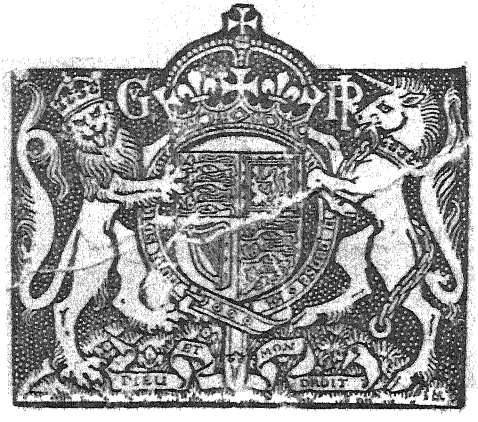


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ERRATA AND AUTHORS' EMENDATIONS

Page 12, fourth line of text from bottom, reference (*35*) should be (*33*).

Page 14, line 7, reference (*44*) should be (*41*).

Page 50, line 22, "oat to hay" should be "oat hay to."

Page 85, table 1, under the heading "Frequency of endodermis pattern" the order of Greek letters should be alpha, beta, gamma. Under the heading "Remarks," the Greek letters should be deleted.

Page 358, twenty-fourth line from bottom, delete the word "less" between "has" and "access."

Page 405, line 16, "¾-inch areas" should be "three quarter-inch areas."

Page 407, table 2, items 18 and 19, "L 3/18" should be "L 3/10."

Page 436, reference 21, "1921" should be "1821."

Page 447, legends for figures 2 and 3, "copper, lime" should be "copper-lime."

Page 448, line 3, "copper, lime" should be "copper-lime."

Page 455, figure 2, "1.91" should be "2.17."

Page 455, fourth line from bottom, "13.4" should be "13.4 percent."

Page 513, equation at bottom should read $\frac{0.2.\ 5.55}{1}=\frac{60-X}{X-40}$

$X=49.470$ F.

Page 621, footnote 3, delete "Underlined values-mean of duplicates."

Page 622, line 17, "table 11" should be "table 1."

Page 624, delete footnote 2.

Page 628, line 8, reference "(*13*)" should be "(*11*)."

Page 687, table 1, column 5, the figure "7" under the variety Hannchen, race 2, should be ".7."

Page 700, line 6, insert footnote reference 3 after weight. The footnote reads as follows: "Later work has demonstrated that the method of drying the residues to constant weight is not applicable to all petroleum oils. It has been found that certain ordinarily used petroleum oils are partially volatile when heated at 110° C. for 2 hours in an electric oven, and this loss may in extreme cases amount to as much as 33 percent of oil recovered from extracted foliage. By substituting the method of evaporating at room temperature under 2 to 3 mm vacuum, as described on page 694 (sentence immediately above table 1), no losses occur. However, the methods as described and without any changes, apply in the analysis of fatty oils like peanut, corn, and cottonseed oil, for it has been demonstrated that no losses occur when these oils are heated at 110° for 2 hours in an electric oven."

Page 724, line 10, "III" should be "3."

Page 727, line 2, "rust" should be "oat."

Page 741, legend for figure 1, "See fig. 8" should be "See fig. 7."

Page 750, table 6, Lot No. 8, under 1929-30, 30° F. column, the "0" should be "0.9."

Page 795, footnote 1, "1937" should be "1938."

Page 811, footnote 1, "1937" should be "1938."

Page 818, plate 1, magnification for D, "× 240" should be "× 540."

Page 820, last paragraph, line 4, "enveloping" should be "developing."

Page 823, table 1, Lot No. 2, column 8, under "Soluble starch" the leaders should be "2.0."

Page 917, table 5, mean revival time, "39.5" should be "38.5."

JOURNAL OF AGRICULTURAL RESEARCH

VOL. 55 WASHINGTON, D. C., JULY 1, 1937 No. 1

EFFECTS OF THE ALKALOID NICOTINE ON THE RHYTHMICITY OF ISOLATED HEART PREPARATIONS FROM PERIPLANETA AMERICANA AND PRODENIA ERIDANIA[1]

By J. FRANKLIN YEAGER, *senior entomologist*, and J. B. GAHAN, *junior entomologist*, *Division of Control Investigations, Bureau of Entomology and Plant Quarantine, United States Department of Agriculture* [2]

INTRODUCTION

This is a report of experiments performed to detect the effects of different concentrations of nicotine, an alkaloid much used in pharmacological studies, upon the contraction rates of isolated insect heart preparations immersed in a perfusion fluid of known composition and temperature, when the pH values of the fluid, with and without dissolved nicotine, fall within the approximate range of 6.8 to 7.8. The isolated heart preparations are so called to distinguish them from hearts truly isolated from their dorsal body-wall connections. They were obtained from large nymphal and imaginal cockroaches (*Periplaneta americana* L.) and from large larval (fifth and sixth instars) southern armyworms (*Prodenia eridania* Cram.).

METHOD

The method used in these experiments is that already described (*40*)[3] and employed (*41*) to detect the cardiac effects of several thiocyanates. It consists in immersing the dissected cardiac preparation, pinned to a wax strip, in air-stirred perfusion fluid in a glass tube held in a water bath having glass sides, through which the heart is illuminated by a strong beam of light. Fresh perfusion fluid from an aspirator bottle is added by drops to that in contact with the heart preparation. Contraction rate is measured with a stop watch or stop clock by determining the time required for 10 complete contractions to occur and converting to beats per minute. Most of the observed points in the illustrative curves represent the average of five such determinations. When the effect of nicotine upon cardiac rate is to be observed, the wax strip is quickly transferred to a second glass tube holding similar air-stirred perfusion fluid in which is dissolved a known concentration of nicotine, and measurement of contraction rate is continued. This process of applying nicotine to the heart will be referred to in this paper as the transfer or strip-transfer procedure. In many experiments a second transfer was made from the saline-

[1] Received for publication Mar. 1, 1937; issued August, 1937.
[2] The authors are indebted to W. M. Mann, director of the National Zoological Park, Washington, D. C., for supplying cockroaches; to L. D. Goodhue, of the Division of Insecticide Investigations, Bureau of Entomology and Plant Quarantine, for purifying the nicotine used in these experiments; and to C. E. Schoenhals, of the Bureau of Plant Industry, and Saburo Katsura for the translation of Yokoyama's article.
[3] Reference is made by number (italic) to Literature Cited, p. 16.

nicotine solution back to the original saline perfusion fluid, after the tissues had been in the nicotine solution from 30 to 60 minutes.

Another method of applying nicotine, to be referred to as the nicotine-addition procedure, has also been used. It consists merely in pipetting a known quantity of strong nicotine solution into a known quantity of perfusion fluid about the heart preparation, thus applying a known final concentration of nicotine to the tissues. In this case no addition of fresh perfusion fluid from the aspirator bottle is made. This procedure eliminates the mechanical agitation of the heart preparations that results from the transfer procedure, but it involves the danger of temporarily applying to the tissues a stronger concentration of nicotine than that desired. To eliminate, or at least minimize, this danger, the nicotine was added slowly by drops during a period of several minutes.

The manner of dissecting out the roach heart preparation has already been described (*40*). In these experiments the head of the roach was removed from the preparation by section through the neck. To dissect out the larval heart preparation, the larva is submerged in a dissecting tray of perfusion fluid and fixed, dorsal surface down, to the wax bottom of the tray by a pin thrust through the head. The posterior end of the body is held in position by grasping one of the anal legs with forceps. A longitudinal incision is made with scissors through the body wall along the dorsopleural line from the posterior end to the anterior end of the body just back of the head. The lateral margin of the dorsal lip of the incision is spread laterally and pinned to the wax. A similar incision is made postero-anteriorly along the dorsopleural line of the opposite side, and, after removal of the entire ventral body wall (sterna) and the attached ventral nerve chain, the posterior dorsal lip is spread laterally and pinned. The dorsal body wall (terga) at the anterior end is spread laterally and pinned and, when necessary to increase the visibility of the heart wall, the middle region is also pinned laterally. The gut is sectioned at its pharyngeal and rectal terminations and is carefully removed as its tracheal connections are cut. The anterior end of the preparation is sectioned back of the head and the head removed. Care is taken to disturb the cardiac mechanism as little as possible during the dissection. The cardiac mechanism is thus exposed to view on the inside of the dorsal body wall, forming the isolated heart preparation. As a rule, in a given experiment a single wax strip held one roach heart preparation and one larval heart preparation; the latter was usually prepared first, and the regurgitated and excreted materials were removed by washing with perfusion fluid before the roach dissection was made.

The perfusion fluid was composed of 9.82 g of sodium chloride, 0.77 g of potassium chloride, and 0.5 g of calcium chloride per liter of solution in distilled water. This is approximately one-eleventh Lévy's (*26*) stock solution minus bicarbonate and phosphate. The buffers were eliminated in order that the nicotine salts formed should be mainly the hydrochlorides. The various concentrations of nicotine were prepared by dissolving the purified alkaloid in the saline perfusion fluid and then titrating with 1 N hydrochloric acid or, in the case of the higher nicotine concentrations, with more concentrated acid, until the pH value of the nicotine solution was the same as that

of the saline solution used for perfusion. The pH values of the saline and nicotine-saline solutions were considered equalized when they yielded the same color with universal indicator (British Drug Houses) upon being compared in the depressions of a white porcelain spot plate.

The concentrations of nicotine used in this investigation ranged from 0.0005 to 10.0 percent by volume. Each experiment was carried out at constant temperature, usually at 29.0° C., and at a perfusion fluid pH value within the approximate range of 6.8 to 7.8, as measured colorimetrically with universal indicator.

In many of these experiments the contraction rates observed before application of nicotine and after transfer of the preparations from the saline-nicotine solution to the original saline perfusion fluid can be considered to serve as controls. A number of control experiments, however, were performed in which no nicotine was used, and the strip transfers were made from saline to saline and back to saline again. Control experiments were also performed in which saline containing no nicotine was applied by the addition procedure.

In most of the experiments contraction rates were measured at shorter intervals than the 15-minute periods used heretofore (*40*, *41*), in order to allow detection of initial rate changes of brief duration. From 15 to 30 minutes usually elapsed between the beginning of dissection and the beginning of perfusion.

Approximately 200 cardiac preparations have been utilized in these experiments.

RESULTS

The results described here were obtained by the use of zero (control), 0.0005-, 0.005-, 0.0125-, 0.022-, 0.025-, 0.043-, 0.044-, 0.050-, 0.086-, 0.088-, 0.10-, 0.17-, 0.177-, 0.20-, 0.34-, 0.355-, 0.39-, 0.40-, 0.69-, 0.70-, 0.71-, 0.77-, 1.37-, 1.42-, 1.47-, 2.69-, 2.75-, 2.85-, 5.5-, 5.67-, 5.70-, and 10.0-percent solutions of nicotine. Unless otherwise specified, the following statements refer to these concentrations.

STIMULATORY EFFECTS

The stimulatory effects of nicotine upon the contraction rates of the cockroach and the larval heart preparations have been found to differ. In the case of the larval southern armyworm preparation the stimulatory effect was relatively small whether the nicotine was applied by the addition (figs. 1 and 2) or the strip-transfer procedure (figs. 3, 4, and 5).

Nicotine stimulation of the roach heart preparation, exhibited as an initial sudden but temporary increase in contraction rate, was observed in most of the experiments involving nicotine application by addition (fig. 1). When the strip-transfer procedure was used, however, these initial changes in contraction rate showed more variation. In general it was found that with the highest concentrations no appreciable stimulation occurred (fig. 5, *C*), that with lower concentrations an initial transient stimulation appeared (fig. 4, *B*, *C*) which might be partially obscured by the occurrence of a transient depression (figs. 4, *A*, and 5, *A* and *B*), and that with still lower and the lowest concentrations used an initial depression was usually obtained (fig. 3, *D* and

F). An initial depression also occurred with zero concentration (control experiments) when the transfer procedure was employed (fig. 3, *A*, *B*, and *C*).

Not only was the cockroach heart stimulated initially by nicotine application, but upon the second transfer back to the original saline solution, even after the heart preparation had remained 30 to 60

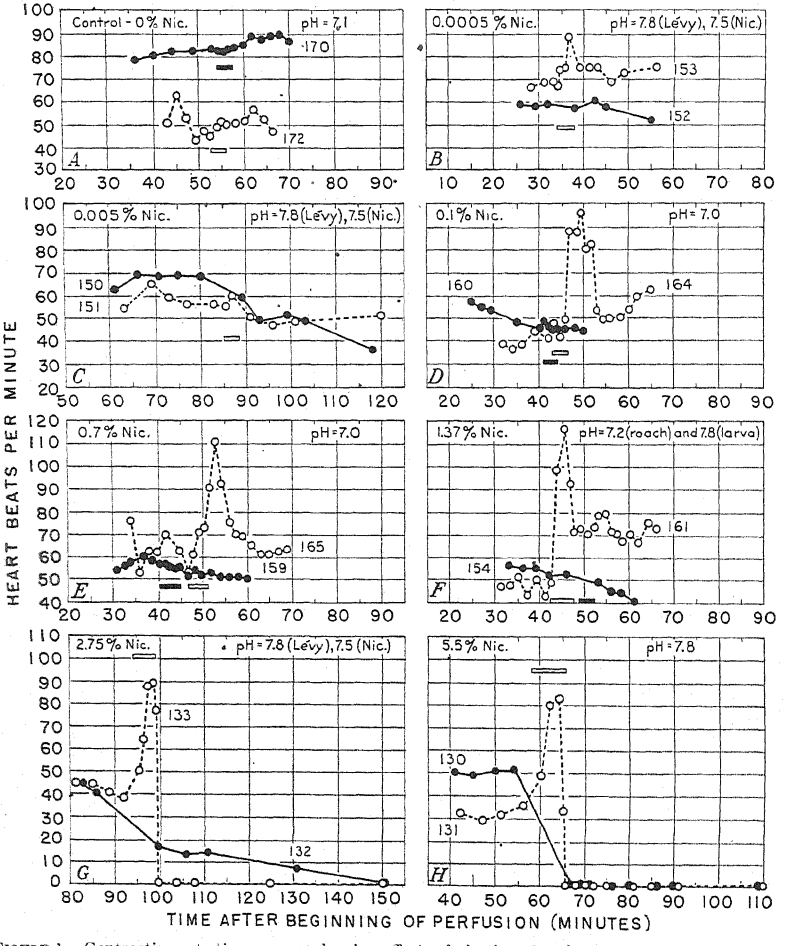


FIGURE 1.—Contraction rate-time curves, showing effects of nicotine of various concentrations applied by the addition procedure to the perfused isolated heart preparations of *Periplyneta americana* and *Prodenia eridania*. Each lettered graph represents an experiment with a different concentration of nicotine or a control. Circles and broken lines represent the roach heart beat and solid dots and lines the larval heart beat. Numbers refer to laboratory number assigned to insect. Small rectangles show the period during which nicotine was being added to the perfusion saline. The pH values indicated are approximate.

minutes in one of the higher nicotine concentrations, it was often left capable of attaining a rate of contraction higher than that exhibited prior to the application of nicotine (figs. 3, *F*, 4, and 5). In many experiments (figs. 3, *E* and *F*, and 4, *A* and *B*), the second transfer was followed by an immediate reversible increase in rate, after which the higher rate level was maintained. In practice, after the second

transfer from nicotine to saline solution, the heart preparation was subject to the action of a very dilute nicotine solution because of the small quantity of the stronger nicotine solution adhering to the strip and attached heart preparation during the transfer.

Initial stimulation of the larval cardiac rate was less marked and of less frequent occurrence than that of the roach heart rate. When the strip-transfer procedure was used, the initial stimulation was usually questionable, but when the addition procedure was used and deter-

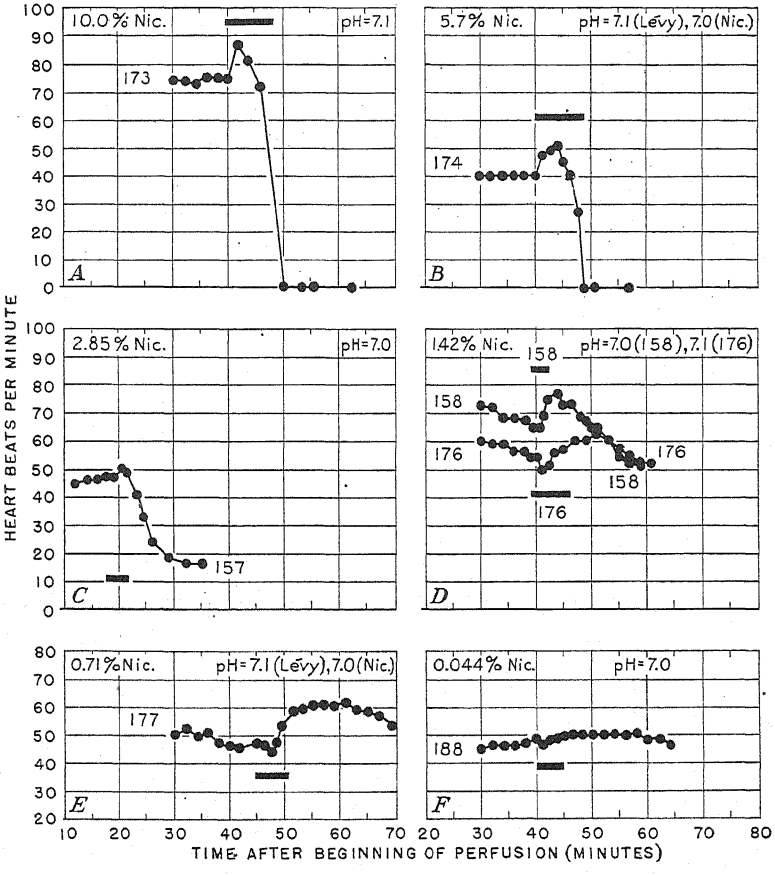


FIGURE 2.—Contraction rate-time curves, showing effects of nicotine of various concentrations applied by the addition procedure to the perfused isolated heart preparation of *Prodenia eridania*. Each lettered graph represents an experiment with a different concentration of nicotine. Small rectangles show the period during which nicotine was being added to the perfusion saline. Numbers refer to laboratory number assigned to insect. The pH values indicated are approximate.

minations of larval heart rate were made in rapid succession, a small initial increase of rate by the higher nicotine concentrations could be detected (fig. 2). In most of these experiments initial stimulation of larval heart contraction rate has not been readily detected.

When roach or larval heart preparation was perfused with saline containing a low concentration of nicotine, even when initial stimulation was not detectable, often the contraction rate trended upward or at-

tained a higher level and the vigor of contraction appeared somewhat increased, giving the impression that dilute nicotine solutions have

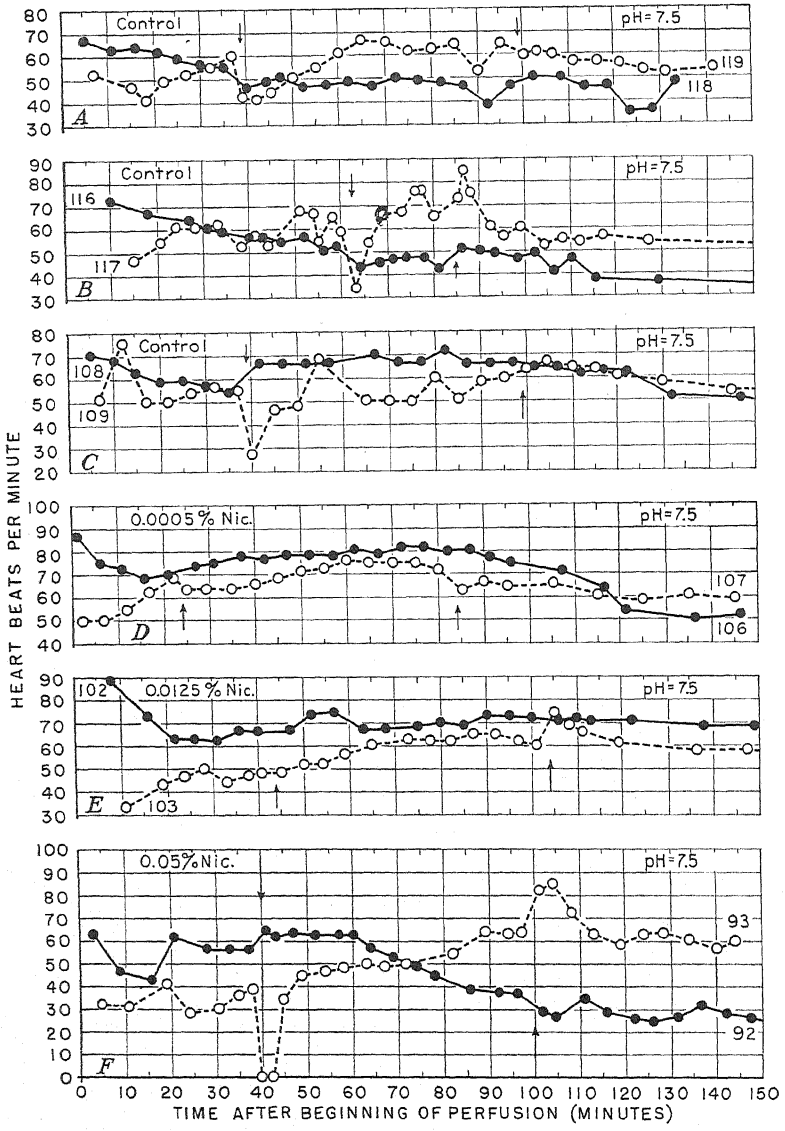


FIGURE 3.—Contraction rate-time curves, showing effects of nicotine of various concentrations applied by the strip-transfer method to the perfused isolated heart preparations of *Periplaneta americana* and *Prodenia eridania*. Circles represent the roach heart beat and solid dots the larval heart beat. Each lettered graph represents an experiment with a different concentration of nicotine or a control. Arrows indicate when the first transfer was made from saline to nicotine-saline solutions and when the second (reverse) transfer was made. Numbers refer to laboratory number assigned to insect. The pH values indicated are approximate.

a beneficial rather than a detrimental effect upon the cardiac mechanism, at least during the period of the experiment.

INHIBITORY EFFECTS

Marked inhibitory effects of nicotine upon the contraction rate of the larval heart were detected in the higher concentrations (figs. 2, *A*, *B*, and *C*, and 5, *B* and *C*). In general this inhibition occurred as a simple decrease in contraction rate to or toward zero, being more rapid and to a lower level the greater the concentration of nicotine (figs. 4 and 5), and it appeared subsequent to initial stimulation (figs. 1, *G* and *H*, 2, *A*, *B*, and *C*, and 4, *C*) when such occurred. Lower nicotine concentrations either had no obvious depressant effect (figs.

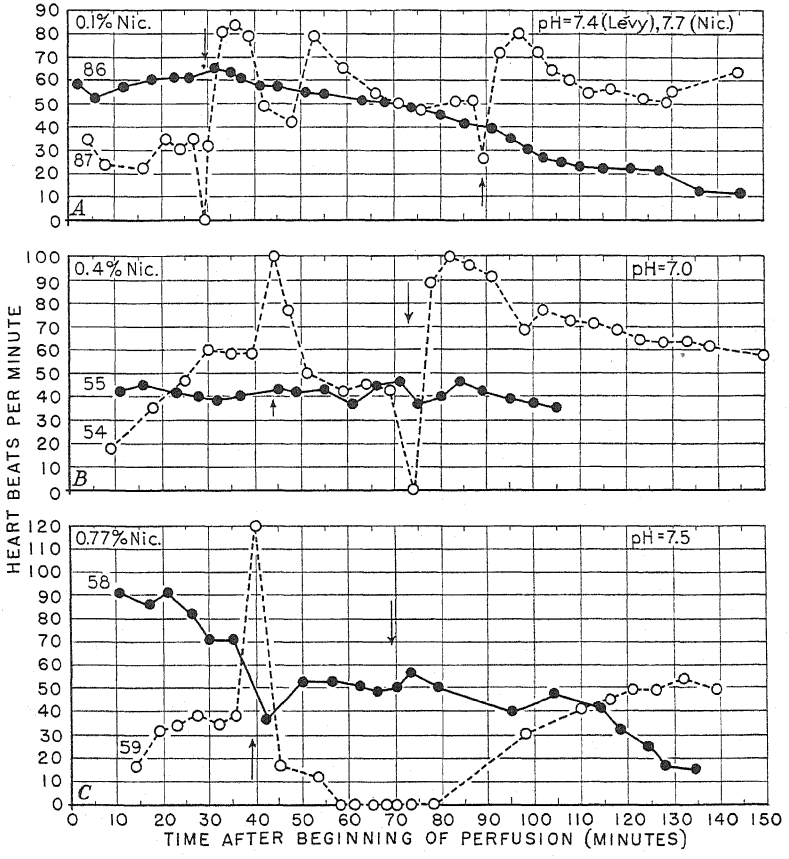


FIGURE 4.—Contraction rate-time curves, showing effects of nicotine of various concentrations applied by the strip-transfer method to the perfused isolated heart preparations of *Periplaneta americana* and *Prodenia eridania*. Continuation of figure 3.

3 and 4, *A* and *B*) or showed a momentary depression of rate at the time of nicotine addition (fig. 2, *D*, no. 176). In all the transfer experiments the inhibitory effects of nicotine on larval heart rate were found to be reversible in the sense that some recovery, as evidenced by an increase in contraction rate, was observed to follow upon the second transfer of heart preparation from nicotine-saline to original saline solution (figs. 4, *C*, and 5).

The contraction rate of the roach heart was inhibited by nicotine at considerably lower concentrations than was that of the larval heart (figs. 1, 4, *C*, and 5, *A* and *B*). In general, when both a stimulation and a depression of contraction rate were detected in a given experiment, the depression, as in the larval heart preparation, occurred subsequent to the initial temporary stimulation, appeared as a more or less rapid decrease in rate to or toward the zero level, depending on the nicotine concentration used, and was reversible in the sense that some degree of recovery followed the transfer from nicotine to original saline (figs. 1, *G* and *H*, 4, *B* and *C*, and 5).

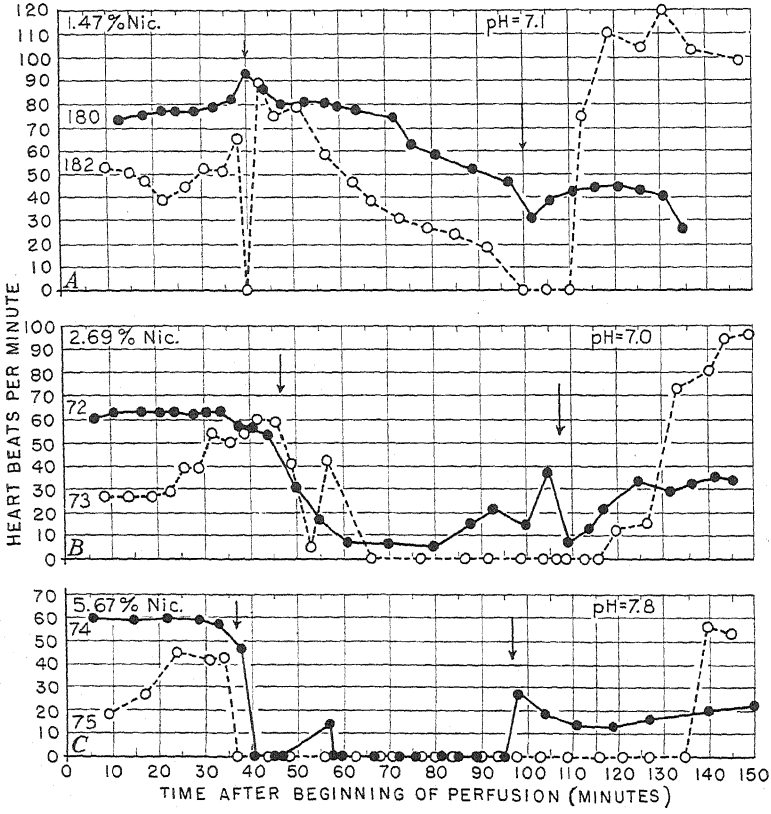


FIGURE 5.—Contraction rate-time curves, showing effects of nicotine of various concentrations applied by the strip-transfer method to the perfused isolated heart preparations of *Periplaneta americana* and *Prodenia eridania*. Continuation of figure 4.

The initial depression of the roach heart contraction rate observed in some of the nicotine concentrations (figs. 3, *F*, 4, *A*, and 5, *A*) when the strip-transfer procedure was used is not considered to be true nicotine depression, since it was also obtained in the control experiments (fig. 3, *A*, *B*, and *C*), although its magnitude may have been influenced by the nicotine. Similar initial depressions have not as a rule been obtained by the addition procedure (fig. 1). In certain nicotine concentrations marked depressions of rate were frequently observed immediately after the second transfer from nicotine to

original saline, prior to the higher recovery level that resulted from the transfer (fig. 4, *A* and *B*).

EFFECTS ON CARDIAC AMPLITUDE

In general, in the case of the larval heart, no decrease of cardiac amplitude appeared to accompany the inhibitory effects of the higher nicotine concentrations. Although a slight increase of amplitude was thought to occur, it was not detected with certainty. Whenever nicotine was observed to reduce the larval cardiac rate to zero, the heart was observed to be in a state of apparent diastolic standstill; at no time in these experiments has nicotine been observed to produce complete systolic arrest of the larval heart.[4]

In the case of the roach heart, amplitude effects of quite a different order were observed to follow the application of nicotine. In those concentrations that gave marked initial stimulation of contraction rate, the quickened rhythm was usually accompanied by an obvious decrease of amplitude in the direction of incomplete relaxation of the cardiac walls, with the result that the rapidly beating heart remained more in systole than in diastole. There was also usually some degree of cardiac incoordination which gave to the heart walls, as they contracted, an appearance of writhing or fibrillation. This fibrillation-like activity was observed to a less marked extent, and as a more transitory effect, in the lower nicotine concentrations, which produced only a slight initial acceleration of contraction rate. Whenever nicotine reduced the roach heart contraction rate to zero within a short time, immediately after cardiac arrest the heart was nearly always found to be in a state of systolic standstill, tightly constricted. Upon the heart preparation remaining in the nicotine solution, however, the cardiac diameter often increased slowly until the heart was neither fully dilated nor fully constricted. Nicotine concentrations too low to cause obvious increase of contraction rate or marked alterations of amplitude seemed, however, to increase the intensity or vigor of contraction.

MISCELLANEOUS OBSERVATIONS

In these experiments it has been noticed that in general the roach heart exhibited somewhat larger and more rapid fluctuations in contraction rate and amplitude than did the larval heart, even before the application of nicotine (figs. 3, 4, and 5).

The contraction rate of the larval heart tended to exceed that of the cockroach heart when the preparations were perfused with nicotine-free saline; in these experiments the average rate of the larval heart was about 58 beats per minute while that of the roach heart was about 49 beats per minute. The rates, however, varied greatly from one insect to another.

Occasionally a condition of heart block was observed in roach or larval heart preparation, more frequently in the latter. The larval heart block occurred sometimes after prolonged perfusion and sometimes with nicotine application. It appeared as one contraction of that portion of the heart lying anterior to the gonads occurring at each second, third, or higher beat of that part lying posterior to the

[4] A larval heart preparation placed in 20-percent nicotine in saline was immediately brought to marked apparent diastolic arrest.

gonads, especially that portion in the rectal segments. In one or two cases the heart block disappeared with cardiac recovery.

In both the roach and larval heart preparations reversal of the direction of cardiac peristalsis was frequently observed. Often the reversals were periodic and occurred fairly rapidly; one roach heart exhibited three or four postero-anterior beats, then three or four anteroposterior beats, and so on, for about 2 hours of observation. Some hearts showed either a postero-anterior or an anteroposterior beat throughout a single experiment, without observed reversal. Between these two extremes occurred varying degrees of reversal periodicity, whether the hearts were in nicotine solution or not. In general, reversals seemed to occur more often in roach than in larval preparations.

That portion of the larval heart lying in the rectal segments usually appeared to be bordered by larger laterally lying masses of tissue than bordered the more anterior regions of the heart. The lateral tissues themselves, in many preparations, exhibited vigorous rhythmic movements, indicating the presence of contained or associated rhythmically contracting muscle fibers. Sometimes the rhythmic activity appeared to be synchronous with the heart contractions, but at other times it was definitely asynchronous. This apparent extracardial rhythm often so interfered with the cardiac contractions, even when their rate continued unchanged, that contraction rates could not be observed in the rectal region but were obtained from the gonadal region. The extracardial activity consisted in the laterally lying tissues being so pulled away from the centrally located cardiac tube as to seem to stretch the latter and interfere with or obscure its contraction.

In many of these experiments diastolic pauses have been observed, usually either during nicotine application or during recovery from nicotine. In many cases apparent irregularities of rate have been caused by occasional diastolic pauses which lowered the average values of the contraction rate at various points on the rate-time curves. Usually when pauses of the nonnicotinized heart occurred, they were diastolic rather than systolic. Systolic pauses were observed frequently in the strongly nicotinized roach heart, sometimes in the nonnicotinized roach heart, but almost never in the larval heart.

Structures that have a superficial resemblance to tubular vessels or channels have been noticed incidentally in the cockroach heart preparations. They extend laterally and at right angles from the region of the heart, especially in the posterior part of the abdomen. During the cardiac cycle they were seen to exhibit rhythmic changes of diameter synchronously or asynchronously with the heart beat, the increase of diameter usually appearing as a more vigorous movement than the decrease of diameter. These apparent vessels or channels occur in pairs, the members of a pair lying on opposite sides of the heart. One member sometimes showed marked rhythmic activity while the other member remained quiescent, though usually both were active at the same time; the activity was persistent or appeared and disappeared several times in a single experiment. Whether these structures are closed tubes or merely open channels has not been determined in this investigation, although movement of liquid in their apparent interior has been observed to accompany their activity. These structures appeared to be the same as the struc-

tures described by Alexandrowicz (*1*) as segmental blood vessels of the cockroach heart.

DISCUSSION

From the results obtained in these experiments it is apparent that, under the conditions described, the cardiac mechanism of the cockroach is considerably more responsive to the action of nicotine than is that of the southern armyworm. The rhythmicity of the latter is stimulated either relatively slightly or not at all and is markedly depressed only by the higher concentrations of nicotine used, whereas the rhythmicity of the roach heart mechanism is stimulated by a wider range of nicotine concentrations and is both initially stimulated and subsequently depressed by the lower concentrations. The roach heart stops in systole and the larval heart stops in partial or complete apparent diastole when arrested by nicotine, and in a given nicotine concentration cardiac arrest occurs earlier in the roach than in the larval heart mechanism. Removal of the preparations back to the original saline solution by a second transfer usually results in an earlier recovery of activity by the larval heart than by the roach heart. The initial rate increase is usually much greater in the roach than in the larval heart preparation. The nicotine-stimulated roach heart often exhibits a fibrillationlike activity that has not been observed in the nicotinized larval heart. Both the rate-stimulatory and the rate-inhibitory effects are essentially reversible processes within the nicotine-concentration range employed in these experiments.

The stimulatory and inhibitory effects of nicotine upon vertebrate and invertebrate hearts have been observed by a number of investigators. One of the more suggestive investigations, from the present standpoint, is that of Carlson (*5*), who studied the effects of nicotine applied separately to the ganglionic and the muscular tissues of the *Limulus* heart. Bringing into consideration other results reported by various authors, he concluded that nicotine affects differently the nervous and muscular tissues not only of the *Limulus* heart, in which anatomical separation of nervous and muscular portions can be readily accomplished, but also of vertebrate and invertebrate hearts generally, in which nervous and muscular elements are not so readily separable. Carlson found that application of nicotine solutions in sea water or plasma to the *Limulus* cardiac ganglia caused a primary (initial) stimulation, exhibited as increased contraction intensity, contraction rate, or both, followed by irregularities of rhythm, depression, or paralysis, depending on the nicotine concentration used; whereas direct application of nicotine solution to the heart muscle had only depressive or inhibitory effects without appreciable associated stimulation, relatively dilute nicotine solutions having no apparent effect. Nicotine concentrations too low to affect the cardiac muscle directly produced marked stimulation when applied to the ganglia. Carlson (*6*) concluded that the action of nicotine upon the vertebrate and the invertebrate heart is primarily upon its intrinsic ganglionic system, and not upon its cardiac muscle or its motor nerve fibers. Carlson's results on *Limulus* bear a suggestive resemblance to the results obtained in the experiments reported in this paper, for the effects of applying certain nicotine concentrations to the *Limulus* heart ganglia and to the cockroach heart preparation are, in both cases, stimulation only (relatively low concentrations) or stimulation followed by more or less rate irregularity, depression, or paralysis

(relatively high concentrations); similarly, application of relatively high nicotine concentrations to the *Limulus* heart muscle and to the southern armyworm heart preparation causes depression or paralysis associated with relatively slight or no stimulation. That a sufficiently high concentration of nicotine can produce stimulation of some striated cardiac or skeletal muscles is indicated by the reports of several investigators (*2, 3, 7, 29, 30*). The earlier investigations of Langley and Dickinson (*23*) and of Langley (*21*) on the effects of nicotine solutions upon autonomic ganglia in the vertebrate indicate that nicotine applied to the ganglia acts primarily upon the ganglionic cells, which become initially stimulated before the onset of depression.

The question naturally arises, Are the differences in effect of nicotine on cockroach and larval heart preparations caused by differences in their intrinsic nervous mechanism? Little research has been reported concerning the intrinsic nervous mechanism of the insect heart. Alexandrowicz (*1*) studied histologically the nervous mechanism of the cockroach ((*Periplaneta*) *Blatta orientalis* L.) heart and reported the presence of ganglionic cells in the lateral heart nerves (nervi cardiaci laterales). Zawarzin (*43*) investigated the innervation of the heart of the *Aeschna* larva and described intrinsic nerve fibers, but found no intrinsic ganglionic cells. Although Dogiel (*12*) reported the presence of what he considered to be Wagner's "birnförmige Körper, oder Nervenzellen" in the cardiac mechanism of the *Corethra* larva, he failed to find nerve fibers extending from them and presented inconclusive evidence of their nervous nature; Walling (*37*) considered these cells to be nonneural in character. Kuwana (*20*) found no ganglia and no ganglionic cells in the heart of the silkworm (*Bombyx mori*). Maloeuf (*28*) failed to detect ganglionic cells in the lateral heart nerves of the dragonfly larva (*Anax junius* Drury). Thus the literature that we do have on the subject would seem to indicate that the hearts of some insects possess intrinsic ganglionic nerve cells whereas those of others probably do not.[5]

The effects of nicotine injected into the vertebrate or applied to the exposed or perfused vertebrate heart are complicated by the possibility of the nicotine's acting upon the extrinsic sympathetic or parasympathetic (vagal) cardioregulatory mechanisms, the intrinsic ganglionic cells, postganglionic fibers, or autonomic motor endings, the auricular-ventricular bundle (Purkinje fibers), the cardiac muscle fibers, or various combinations of these. It is not surprising, therefore, that various authors have obtained results that are seemingly not in accord as regards the cardiac effects of nicotine. It has been indicated that nicotine may influence heart action by affecting the vagal cardio-inhibitory center (*18*), the intracardiac ganglia (*8, 19*), the endings of the motor fibers (*29, 35*), and the cardiac muscles (*5, 6, 29, 30*). A similar complexity is no doubt involved in the effects of nicotine on the insect or other invertebrate heart that is controlled by extrinsic and intrinsic nervous mechanisms.

[5] It is of incidental interest in this connection that Steiner (*35*) and Fries (*14*), applying the method of critical thermal-increment analysis, reported indirect evidence that the heart beats of the cockroaches *Periplaneta americana* and *Blatta orientalis* are under neural control. Garrey (*15*) reached a similar conclusion concerning the *Limulus* heart from a study of the temperature coefficients of whole heart and separated cardiac ganglia. Crozier and Federighi (*11*) arrived at a similar conclusion regarding the heart beat of the silkworm. Even though the thermal-increment analysis method may yield indirect evidence that the respective cardiac activities are under control of nervous tissue, it alone (i. e., in the absence of additional experimental investigation) does not indicate the locus of such neural control. This applies especially to the experiments of Fries (*14*) and Crozier and Federighi (*11*). Steiner (*35*), who used additional experimenta procedures, suggested that the ganglionic cells of the roach's lateral heart nerves constitute the source o neural control of its cardiac activity.

With these observations in mind, it is obvious that a complete explanation of the results reported here is not readily attainable. Nevertheless, it seems probable that with further acquisition of knowledge of the insect heart these results may receive a reasonable explanation in one of the following ways:

(1) The hypothesis can be formed that, although in the cockroach heart preparation the applied nicotine acts upon a cardiac mechanism possessing intrinsic ganglionic cells, in the larval heart preparation it acts on a mechanism lacking ganglionic cells. In accordance with this explanation, the nicotine would affect primarily the ganglionic cells of the roach heart with initial stimulation and subsequent depression and in the larval heart would act primarily upon the intrinsic nerve fibers, their motor endings, or the cardiac musculature, all of which, in the vertebrate, have been shown (*5*, *6*, *21*, *22*, *23*, *24*, *31*, *33*) to be less readily affected, especially in an excitatory manner, by nicotine than are ganglionic cells.

(2) It may be supposed that both heart mechanisms possess intrinsic ganglionic cells but that these cells, the cardiac muscles, and the intrinsic nerve fibers and nerve endings have relatively different resistances to the action of nicotine.

These two possible explanations are presented as working hypotheses, with the view that additional anatomical knowledge regarding the existence of intrinsic ganglionic cells in the two cardiac mechanisms should offer evidence tending to confirm or refute the one or the other.

The effects of nicotine solutions on cardiac amplitude in the roach heart preparation are in general accord with those reported by other investigators who have used hearts possessing intrinsic ganglionic cells. Clerc and Pezzi (*10*) report that auricular fibrillation and flutter, as well as other abnormal cardiac phenomena, can be detected in the dog heart following the injection of nicotine; the fibrillation is attributed (*9*) to the action of nicotine on vagal innervation. Levy (*25*) observed ventricular fibrillation of the anesthetized cat heart subsequent to nicotine injection. Burridge (*3*) found that perfusion of the frog heart with a sufficiently high concentration of nicotine caused cardiac contracture with loss of relaxation capacity. Barry (*2*) observed increased intensity of contractions, appearance of extrasystoles, shortening of the auricular-ventricular interval, and double auricular rhythms among the effects produced by nicotine on the perfused frog heart. The investigations of Langley (*22*) show that nicotine contraction and nicotine contracture [6] are producible in muscle fibers (skeletal), and that the regions of the neural endings are more sensitive to the drug than are the nonneural regions. Although the investigations of these and other authors indicate that nicotine can increase the degree of skeletal and cardiac muscular contraction either by direct action on the muscle fibers or by indirect action via their nerve supply, or both, the mode of action of nicotine in producing the fibrillationlike activity and systolic arrest of the roach heart is not known. It is probable that both an indirect neural and a direct muscular action occur as the concentration of nicotine in the cardiac tis-

[6] "Contracture" is a poorly defined term for an incomplete, reversible, nonpropagated mechanical shortening of muscle not necessarily accompanied by the propagated action current typical of contraction. "Contraction" refers to the usual twitch and tetanus type of muscular response, well known in muscle physiology. Often contracture is used with the meaning of prolonged shortening.

sues is built up, and that the final systolic arrest of the roach heart indicates a direct action of nicotine on the cardiac muscle fibers.

The role of the alary muscles in the insect cardiac cycle and in the cardiac effects of nicotine is not clearly understood. Suggestions have been made that the alary muscles actively participate in diastole (*38, pp. 458, 460*) and that they more or less passively affect the dilation of the heart by slight changes in tension (*44*). Mechanically, insofar as the alary and cardiac muscle tensions are concerned, the insect heart, arrested by nicotine or any other cause, should stop in systole or diastole depending, respectively, upon the less or greater tension of the alary than of the cardiac muscle fibers at the time of cardiac standstill. The present observations indicate that at the time of nicotine arrest the relative tension of the roach cardiac muscle becomes increased (nicotine contracture?). If the cardiac muscle tension of the nicotine-arrested larval heart is also increased, then, since the larval heart does not stop in apparent systole, the tension of the alary muscles must be increased to a relatively greater extent. Federighi (*13*) has found that the peristaltically rythmic dorsal vessel of the annelid *Nereis virens* Sars possesses smooth muscle fibers and is brought to diastolic arrest by nicotine. Zilwa (*44*) has also reported results indicating that nicotine can directly or indirectly decrease the tension of smooth muscle. Barry (*2*) found that the auricles of the perfused frog heart are brought to diastolic standstill by nicotine, although the ventricle of the same heart passes into systolic arrest. The failure of nicotine, even in very high concentration, to produce systolic arrest of the larval heart would seem to indicate either that the cardiac muscle of the southern armyworm is different not only from that of the roach used here but also from the cardiac and skeletal muscle known to exhibit nicotine contracture, or that the larval alary muscles are thrown into contracture of intensity sufficient to overcome that of the cardiac muscle and thereby to produce apparent diastolic standstill.

The initial stimulating action of the lower nicotine concentrations is readily detected by the nicotine-addition procedure, but when the transfer procedure is used this action is partially or entirely masked by the inhibitory effect of the transfer, which occurs in the control experiments, especially in the case of roach heart preparation. This transfer depression of rhythmicity probably results from the mechanical stimulation of the heart mechanism.

The pH values of the saline and nicotine solutions used for perfusion were kept within the approximate range of 6.8 to 7.8, and in a given experiment were equalized or nearly equalized. At one end of this range (pH 6.8) the ratio of undissociated (alkaloid) nicotine (Nic HOH) molecules to the dissociated nicotine ions (Nic H^+) attains the theoretical value of approximately 1:2.4, and at the other end (pH 7.8) the ratio is about 1:63. These values are of the applied solutions, and not of the solutions after penetration into the cardiac tissues where their pH values may become subject to alteration by tissue buffers, especially in the early stages of perfusion; nor is account taken of the nicotine salt (hydrochloride) that is formed in the nicotine-saline solution as a result of the presence of ionizable electrolytes. In any case the dissolved nicotine may be considered to be from about 40 to about 1.6 percent in un-ionized form, while presumably a considerable portion of the ionized nicotine becomes the hydro-

chloride salt. It is to be expected that these conditions would necessitate the use of relatively higher total concentrations of nicotine to affect the cardiac tissues than if the nicotine had been used in unneutralized solution, since several investigations (*6*, *23*, *27*, *32*) indicate that nicotine is more effective when applied to tissues in alkaloid than in salt form.

It has been observed incidentally in these experiments that, if certain nicotine concentrations that do not arrest the perfused larval hearts are injected into intact larvae, the larvae can be quickly and completely paralyzed, although their intact hearts continue to pulsate. It would appear, therefore, that the cardiac tissues of these insects, especially the southern armyworms, possess higher resistance to the action of nicotine than do the intact insects. Shafer (*34*) noted that cardiac activity was one of the last visible indications of life in insects killed with various poisons, and Walling (*36*) observed that various gases decreased cardiac activity of the grasshopper less readily than respiratory activity.

The causes of the large nonnicotine fluctuations in rate shown by most of the rate-time curves, including those from the control experiments, are not known. They have occurred throughout these experiments and can be observed in contraction rate-time curves of intact insect hearts presented by other investigators (*4*, *39*). Thus, both the isolated and the intact insect hearts exhibit variations of this sort, at least when measured by stop watch. It should be emphasized that the contraction-rate values obtained in these experiments and in the experiments of many other investigators are average values representing given contraction periods, and therefore give no indication of the variability of the individual beats that lie within those periods and tend to give inexact representations of some cardiac phenomena, such as extrasystoles and dropped beats. Stop-watch measurements of contraction rate yield only a portion of the desired information concerning the effects of nicotine on the insect (or other) heart. It is known that nicotine acting on the noninsect heart can produce changes in amplitude as well as in rate. This has appeared to be true in some of the present experiments in which low concentrations of nicotine seem to increase cardiac vigor. In the experiments reported here, therefore, only that portion of the nicotine action upon the roach and larval hearts which is exhibited as alteration of contraction rate has been measured.

The causes of reversal in the isolated heart preparations are not known. Yokoyama (*42*) has presented evidence that reversal in the silkworm heart is caused, partly at least, by factors such as oxygen supply, temperature changes, or applied adrenalin, which produce increases or decreases of automatic rhythmicity at one end of the heart relative to the other end. Gerould (*17*) has also produced cardiac reversals by altering the oxygen supply to the intact insect heart, but he has also observed (*16*) reversals in the isolated heart preparation. It seems doubtful that variations in oxygen supply caused the reversals observed in these experiments, since reversals occurred under conditions of continuous aeration of the perfusion fluids; nor, since reversals occurred in nicotine-free saline, are they entirely attributable to the alkaloid. It is of interest, nevertheless, that Barry (*2*) obtained ventricular-auricular rhythms, as well as other coordination disturbances, in the nicotine-perfused frog heart.

SUMMARY AND CONCLUSIONS

Isolated heart preparations from the fifth- or sixth-instar southern armyworm (*Prodenia eridania* Cram.) and the American cockroach (*Periplaneta americana* L.) have been perfused with various concentrations of nicotine to determine the effect on cardiac contraction rate. Two methods of perfusion have been used, the strip-transfer procedure and the nicotine-addition procedure, and the results and conclusions drawn apply only to the above-named insects and under the specified conditions of nicotine application.

The transfer procedure does and the addition procedure does not result in a frequently occurring initial cardiac depression of rate, which is considered to be nonnicotinic in origin.

In general, with regard to contraction rate, nicotine applied to the roach and larval heart preparations causes initial stimulation without depression at relatively low concentrations, initial stimulation followed by partial depression at relatively intermediate concentrations, and stimulation followed by complete depression and paralysis at relatively high concentrations.

The magnitude of the effect of nicotine upon contraction rate is in general much greater in the roach than in the larval heart preparation.

The stimulatory and inhibitory responses of the cardiac mechanism are elicited by relatively lower nicotine concentrations applied to the roach than to the larval heart preparations.

Both the stimulatory and the inhibitory effects are essentially reversible.

The nicotine-arrested roach heart is brought to systolic standstill, whereas the nicotine-arrested larval heart is brought to partial or complete apparent diastolic standstill; in general, the pulsating roach heart does and the pulsating larval heart does not tend toward greater apparent systole under the action of nicotine.

The roach heart can be stimulated by nicotine to exhibit a fibrillationlike activity that has not been observed to result from similar stimulation of the larval heart.

The roach and larval hearts may exhibit considerable fluctuation of contraction rate whether under the influence of nicotine or not; the fluctuations tend to be more marked in the roach than in the larval heart.

The roach and larval hearts may exhibit reversals of direction of pulsation (reversals of beat) whether or not they are under the influence of nicotine.

It is suggested that the marked differences in response of the roach and larval isolated cardiac mechanisms to perfusing nicotine may be due to differences either in their possession of intrinsic ganglionic cells or in the resistance to nicotine of various of their component neural or muscular parts.

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THE BEET LEAFHOPPER IN THE CENTRAL COLUMBIA RIVER BREEDING AREA [1]

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INTRODUCTION

The Yakima Valley in Washington is a potential sugar-beet-producing area, but production is limited by the prevalence of curly top disease, caused by the beet leafhopper, *Eutettix tenellus* (Baker). Three sugar factories built before the severity of this disease was realized have now been dismantled. An attempt to produce white pea beans commercially in the vicinity of Walla Walla in 1930 was abandoned, apparently for the same reason, and tomato growers there suffer heavy losses almost every year. In fact, susceptible crops in vegetable gardens throughout this section are severely damaged.

Surveys conducted by the Bureau of Entomology from 1927 to 1929 [3] indicated that there is a large breeding area of the leafhopper in the central Columbia River Valley, in south-central Washington and northeastern Oregon, from which populations are dispersed to the Yakima Valley and other surrounding territory (fig. 1).[4]

In 1930 ecological studies of the beet leafhopper were undertaken in this area. A laboratory was established at Hermiston, Oreg., and 24 stations were set up for the purpose of making observations on this insect. These stations were so placed as to include the more important host plants and were widely enough separated to be representative of the entire breeding area. To determine the host-plant sequence, brood occurrence, and population fluctuations throughout the season, observations were made at these stations as nearly as possible every 10 days from spring to fall over the period 1930 to 1933. Population sampling was accomplished the first year by means of the sweep net, supplemented by square-yard counts in the spring and late in the fall, when the temperature was so low and host plants were so small as to make sweeping impracticable. The square-yard counts were made by covering a definite area on hand and knee and counting the insects disturbed. The square-foot sampler,[5] a cylindrical cage with a 5-foot handle and covering 1 square foot of soil surface, was devised in the spring of 1931, and was used exclusively after that time.

LIFE HISTORY

The beet leafhopper overwinters in the central Columbia area only as the fertilized female. During the year there are at least three broods. The spring brood appears in May, the summer brood about

[1] Received for publication Mar. 1, 1937; issued August 1937.

[2] The writer's thanks are due P. N. Annaud, formerly in charge of this project, for guidance and suggestions in the study of this problem; and to W. C. Cook for helpful suggestions in the preparation of the manuscript.

[3] These surveys were conducted by H. E. Wallace, C. H. Griffith, and the writer.

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the first of July, and the fall brood late in August (fig. 2). The spring and summer broods overlap, causing the peak of populations for the

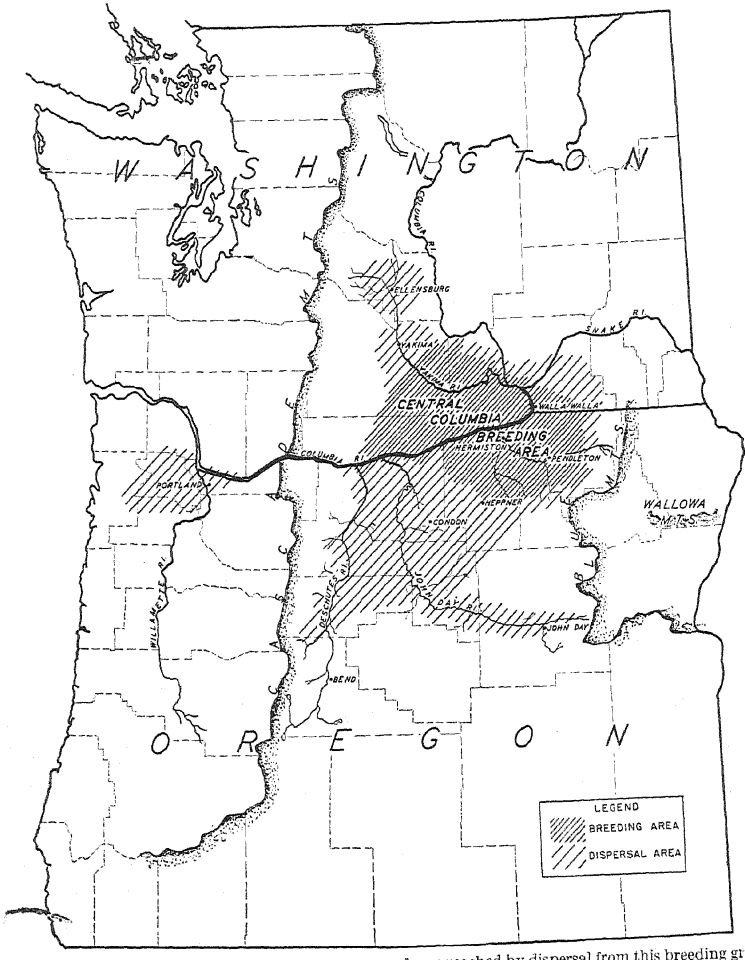


FIGURE 1.—Central Columbia River breeding area and area reached by dispersal from this breeding ground.

season to come in July or August. There is also an overlapping of the summer and fall broods, but host-plant and weather conditions

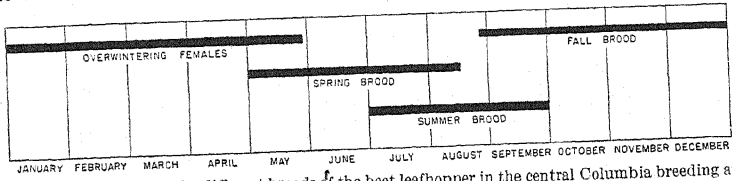


FIGURE 2.—Occurrence of the different broods of the beet leafhopper in the central Columbia breeding area.

at this time are such that populations are not so high as during the summer months.

HOST PLANTS

The principal overwintering host plants of *Eutettix tenellus* in this area are alfileria (*Erodium cicutarium*) and tumblemustard (*Norta altissima*). This mustard is also the most important host of the spring brood. Russian-thistle (*Salsola pestifer*) is by far the most important host of the summer brood. The fall brood matures on Russian-thistle, but the drying of this plant forces the insects to shift to the young fall-germinated plants of mustard and alfileria.

HOST OF THE SPRING BROOD

Spring-brood nymphs hatch and develop largely on tumblemustard and alfileria. These plants germinate in the fall and are usually abundant early in the spring. The tumblemustard usually lives longer than the alfileria, but since the two occur in close proximity or in intermixed stands the insects easily shift from alfileria to tumblemustard. Ordinarily neither plant dries before most of the spring brood matures, and many of the eggs are deposited in the mustard. Each year summer nymphs have hatched from the mustard just as it dried.

Tumblemustard is subject to considerable competition by downy bromegrass (*Bromus tectorum*). Much of the area in which tumblemustard was dominant in 1930 was entirely replaced by bromegrass in 1933. Alfileria is not so easily crowded out by the bromegrass, since it grows much faster late in the fall and early in the spring than does either tumblemustard or bromegrass. Moisture is sufficient for both plants at these times, and alfileria usually matures by the time the bromegrass becomes large enough to crowd it out. The tumblemustard, maturing later, comes into direct competition with the bromegrass at a time when moisture is scarce.

Large acreages of tumblemustard were noted to have succumbed to drought since 1929. In the summer of 1929 this mustard was abundant over much of the area between the Columbia and the Yakima Rivers. In 1930 young plants were observed the middle of March, but they had dried by the middle of April. Very few plants were seen in 1931, and most of these dried before reaching maturity. As a result the area was almost barren of tumblemustard during 1932 and 1933.

In 1932 *Pectocarya penicillata* harbored large populations of spring-brood nymphs and adults. Previously the plant had not been noticed. Its distribution was limited, and it was found to be relatively short-lived. In 1932 its seeds germinated late in February and the plants were mature and dry by May; in 1933 the seeds germinated the first week in March and the plants had matured and dried by the first week in June. In most places many large nymphs of the spring brood were present at the time the plants dried.

Bassia hirsuta, though of limited distribution, harbors rather large populations of the spring brood. It germinates either in the fall or spring and remains green until the following fall. Germination was noted on October 27, 1930, November 18, 1932, March 7, 1931, and March 14, 1933.

Chenopodium leptophyllum is a host of minor importance. Prior to 1933 population counts had not been made on this plant owing to its limited distribution. In 1933 it was slightly more abundant, but spring-brood populations on it were comparatively small.

HOSTS OF THE SUMMER BROOD

Russian-thistle, the most important summer host, has germinated in March every year, but it has not harbored large populations until after the spring dispersal. The entire life cycle of the summer brood is spent on this plant.

Gilia minutiflora is primarily a host of the summer and fall broods. The seeds usually germinate in April and the plants do not dry until late in October. Where *Gilia* is intermixed with tumblemustard, as it often is, populations on it are small until the mustard becomes unsuitable; where it occurs away from spring hosts, populations prior to dispersal are small. Populations have never been known to be large on this host, but in some years its abundance has made it important. In 1930, when it first attracted attention as a host of *Eutettix tenellus*, it was very abundant. That summer it was completely defoliated by the larvae of *Heliothis* sp. Later the plants leaved out and bloomed to a limited degree, but before they could set seed they were again defoliated, this time by a second brood of *Heliothis*. The plants were so completely prevented from setting seed by these two broods of *Heliothis* that *Gilia* was not again abundant until 1933, and even then it was less so than in 1930.

Chenopodium leptophyllum is a minor host of the summer as well as of the spring brood, since it does not usually dry until September.

HOSTS OF THE FALL BROOD

Host plants of the fall brood are of three distinct types: (1) Those on which the brood hatch and develop, (2) those on which the females overwinter, and (3) those that furnish food and a medium for oviposition the following spring. Of the first group Russian-thistle is by far the most important, with some breeding occurring on *Gilia minutiflora*, *Chenopodium leptophyllum*, and *Bassia hirsuta*. Alfileria is the most important in the second group owing to its abundance, while tumblemustard is the most important in the third group. This mustard germinates early in March and large spring populations of nymphs indicate that it is preferred for oviposition by the overwintered females.

SEASONAL OCCURRENCE

The magnitude of the spring brood of *Eutettix tenellus* depends upon the numbers of overwintered gravid females present in the spring. Thus, fall drought, type of winter, or any other factor that affects the overwintering of the fall-brood females has a marked effect on spring-brood populations. The time of appearance of this brood depends largely upon the temperature.

As will be seen from figure 3, spring-brood nymphs appeared each year about the first of May [6] and reached the peak of abundance the latter part of May. The low level the middle or latter part of June represents the end of that brood. The curves did not reach zero in June because of the hatching of the summer-brood nymphs and consequent overlapping of broods.

In 1931 an abundance of spring nymphs was expected because of the relatively large populations of overwintered females (fig. 3) and

[6] The data for 1930 were obtained by sweep-net sampling and are in terms of insects per 50 sweeps of a 14-inch net. They are not comparable to those obtained from the square-foot samples and are therefore not presented in figure 3.

the early egg development (fig. 6). Such is not indicated in the curve for that year. This inconsistency was probably due to imperfections in the technique of removing and counting small nymphs, since it was the first spring the counting cage was used. Sweep-net samples and the early appearance of large numbers of spring-brood adults indicated that nymph populations were comparatively large during May 1931.

In 1930 and 1931 spring-brood adults appeared from the middle to the latter part of May, populations reaching their peaks in June and declining after July 1, but in 1932 and 1933 they did not appear until about June 1, reaching their peaks the last of July. In figure 3 disappearance of the spring-brood adults cannot be determined, since the summer brood appeared about the first of July and caused overlapping.

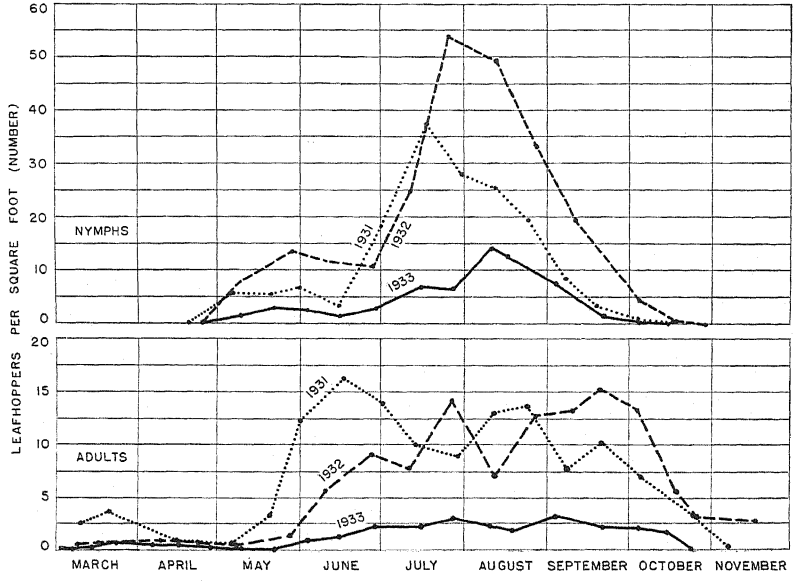


FIGURE 3.—Mean populations of the beet leafhopper in the breeding grounds.

Each year there has been a sudden increase in nymph populations and an appearance of very small nymphs late in June, which probably denotes the hatching of the summer brood. The summer and fall broods greatly overlap, so that figure 3 scarcely shows where one leaves off and the other begins. The slight decrease toward the end of July probably represents the beginning of the decline of the summer-brood nymphs. The time of appearance of the summer-brood adults is masked by the presence of the spring-brood adults. The peak of summer-brood adults came in August in 1930 and 1931, and the first of September in 1933. In all probability the 1932 peak also came the first of September, although this is not shown in figure 3, because the early appearance of large numbers of fall-brood adults undoubtedly prevented a drop in September populations.

The time of hatching of the fall-brood nymphs cannot be determined from figure 3, but it is undoubtedly the hatching of this brood that prevents the curve from dropping to zero in August, as the trend

the latter part of July would indicate. Figure 3 shows a peak in September for fall-brood adults in 1931 and 1932. In 1933 populations were small, and in September fall-brood emergence scarcely replaced summer-brood mortality. Therefore, no fall-brood peak is shown for this year. The definite drop in adult populations in October is caused by the drying of the summer hosts, principally Russian-thistle. The last point in the 1931 curve for adults and the last two points in the 1932 curve represent collections on newly germinated mustard and

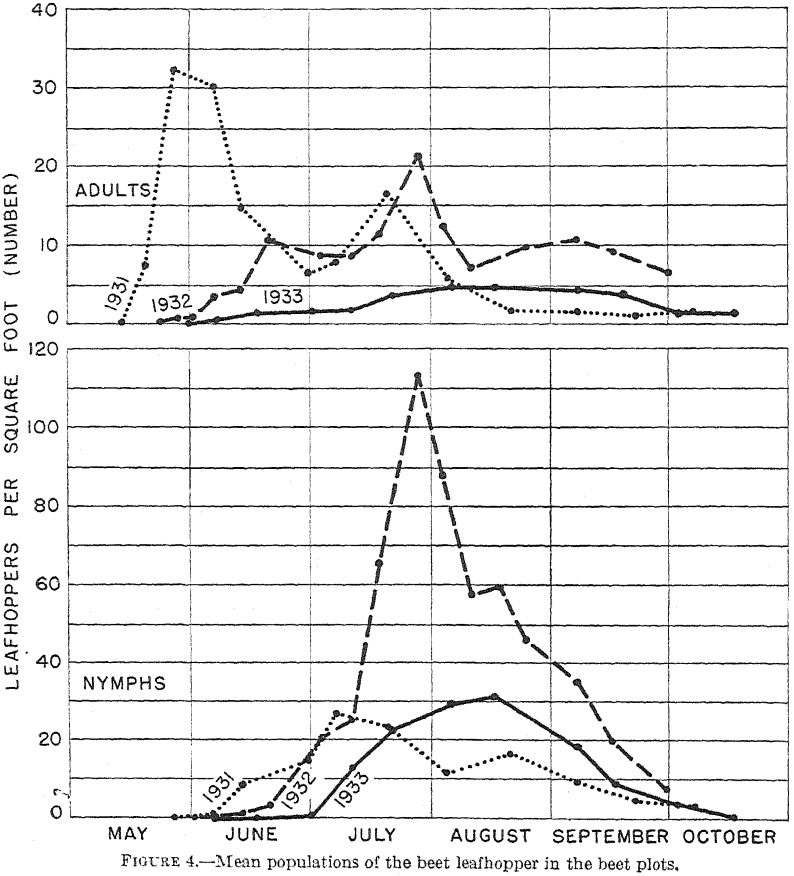


FIGURE 4.—Mean populations of the beet leafhopper in the beet plots.

alfileria. As fall hosts cover a much larger area than summer hosts, the shift results in a decreased population density, but there is probably a real mortality due to the forced shifting. The small fall populations shown are a resultant of both these causes.

DISPERSALS

Movements or dispersals of the spring brood have been studied by observations in beet plots and by catches in insect traps. Since no sugar beets are grown in the central Columbia area, one-fourth-acre plots were planted to beets in cooperation with the farmers for the

purpose of studying *Eutettix tenellus* populations as well as disease conditions. Three were planted in 1931, three in 1932, and four in 1933. Prior to the spring dispersals the beet plots contained only a few overwintered females, which had no doubt come from adjoining wild hosts. Therefore, the increase in adult populations during May and June (fig. 4) was due entirely to an influx from the breeding ground. The curves for adults in figure 4 indicate both the time and the relative magnitude of the dispersals for these years.

A comparison of figures 3 and 4 shows that while adult populations are large in the breeding grounds at the time of migration, the peak is not reached until migration is over. It is possible that migration is so closely associated with brood maturity in the breeding grounds that the peak of adult populations is prevented until after migrations are over.

Approximate dates of spring dispersals as determined by windvane trap [7] collections and beet-plot population studies are as follows:

1931	May 19–20, 25–30. June 1–12. July 6–15 (slight).
1932	May 25 and 31. June 1–25. July 1–5 (slight).
1933	June 1–19 (slight).

In 1931 four traps were placed on the city water tower at Stanfield, Oreg., at heights of 38, 74, 108, and 127 feet above the ground, to determine the relative numbers of insects at those heights. During the season 18 *Eutettix tenellus* were caught in the trap at 38 feet, 12 at 74 feet, 6 at 108 feet, and 10 at 127 feet. Although these data are meager, they indicate that the flying insects are most numerous close to the ground.

The records obtained indicate that large populations in the breeding ground precede heavy dispersals, and since it is the spring brood that disperses to the cultivated area, the magnitude of this brood in the breeding ground is of prime importance. Figure 3 shows that the spring-brood populations in the breeding grounds were much larger in 1931 than in 1932 and 1933. Likewise, figure 4 shows a much larger spring-brood population in the beet plots in 1931 than in either 1932 or 1933. Curly top was severe in the plots in 1931, but not in 1932 and 1933. Curly top injury to tomatoes over the Umatilla irrigation project was also more severe in 1931 than in either 1932 or 1933. The magnitude of the summer brood in the beet plots, as indicated by the July peaks in figure 4, is of comparatively little importance, since the older plants are more resistant to the disease.

FALL AND WINTER SURVIVAL

Survival of the fall brood may be divided into two periods, fall and winter. Fall survival is largely dependent upon proper host-plant sequence. A change of host is necessitated by the maturation of Russian-thistle, which usually occurs from September 15 to October 15. High fall survival is favored when rainfall is sufficient to germinate winter hosts before Russian-thistle matures. Fall survival is low when fall rains are late and germination is delayed until after

[7] FULTON, F. A., and CHAMBERLIN, J. C. A NEW AUTOMATIC INSECT TRAP FOR THE STUDY OF INSECT DISPERSION AND FLIGHT ASSOCIATIONS. Jour. Econ. Ent. 24: 757–761. 1931.

Russian-thistle reaches maturity. When this occurs the insects are forced to feed on unfavorable food plants until winter hosts germinate.

In the period covered by these studies four distinct types of winter were represented. During January of the winter 1929–30 there were 16 days of subzero weather with a minimum temperature of −38° F., accompanied by 15 inches of snow. Snow was on the ground 5 days during December and 19 days during January. During the winter of 1930–31 there was no snow and the minimum temperature was 15°. The winter of 1931–32 set in early. The temperature dropped to 8° on November 21 and was consistently low from then to the middle of December, with a minimum of 2° on November 28. There were 5 inches of snow on the ground during this period. Temperatures during the last half of December and all of January were moderate, but from February 1 to 5 they were below 0°, with a minimum of −20° on February 2. The soil-surface temperature on this date was 26°. These low temperatures were accompanied by 8 inches of snow. From November 25 to February 10 snow was on the ground for 52 days. During the winter of 1932–33 there were 7 days of subzero temperature, with a minimum of −9° in December; there was no snow cover during this period, and the soil-surface temperature dropped to 2°. On February 9 the minimum air temperature was −5° and, with no snow cover, the soil temperature dropped to 5°.

The winter of 1932–33 is the only one of the four which had any apparent effect on leafhopper survival. Field counts taken in fall and spring, as well as hibernation-cage results, show that the winters of 1929–30, 1930–31, and 1931–32 had little if any effect on the insects, whereas only 4 percent of the insects survived the winter of 1932–33. The extremely low soil-surface temperatures affected the insect not only directly but indirectly by limiting the food supply, since practically all alfileria and tumblemustard were killed.

SEASONAL COLORATION

Severin [8] mentions certain differences in color patterns of *Eutettix tenellus* in California. These seasonal color patterns have been found to occur in Oregon. In general there are three color types, the dark forms, which are the usual overwintering type, and the green and straw-colored forms, which occur during the spring and summer. In 1931 and 1932 *E. tenellus* adults taken in the regular population counts were classified according to color (fig. 5). The difference in coloration between the green and the straw-colored forms during the spring and summer is not always clear-cut, but there is always a noticeable difference between the spring and summer forms and the dark overwintering forms. This difference in coloration may or may not be associated directly with brood occurrence, but it will be noted from figure 5 that early in the spring, when only overwintered females are present, only the dark forms occur. The sudden increase in May or June is due entirely to the green forms. The peak of the summer brood is caused largely by the straw-colored forms, and the fall populations are made up almost entirely of straw-colored and dark forms. The fall and winter mortality of the straw-colored forms is very high, so that by spring the populations consist almost entirely of dark forms.

[8] SEVERIN, H. H. P. LIFE-HISTORY OF THE BEET LEAFHOPPER, EUTETTIX TENELLUS (BAKER), IN CALIFORNIA. Calif. Univ. Pubs. Ent. 5 (4): 37–88, illus. 1930.

EGG DEVELOPMENT

A special technique was devised for studying seasonal ovule development. About every 10 days samples of females were taken from a locality in which the host-plant sequence was adequate to maintain the insect throughout the year. These samples were preserved in a 5-percent aqueous solution of chloral hydrate. Twenty-five females for each date of collection were dissected under a binocular microscope, and then measurements were made by substituting a sliding-scale ocular micrometer for one of the oculars. Comparative development was determined by measuring the developing ovules within the female, and egg length was taken as an indication of the degree of development. Only ovules over 0.20 mm in length were recorded, smaller ovules being considered unimportant for this work. Results were expressed in terms of total length of all eggs over 0.20 mm for each female, and the mean total length of eggs for each sample was taken to represent

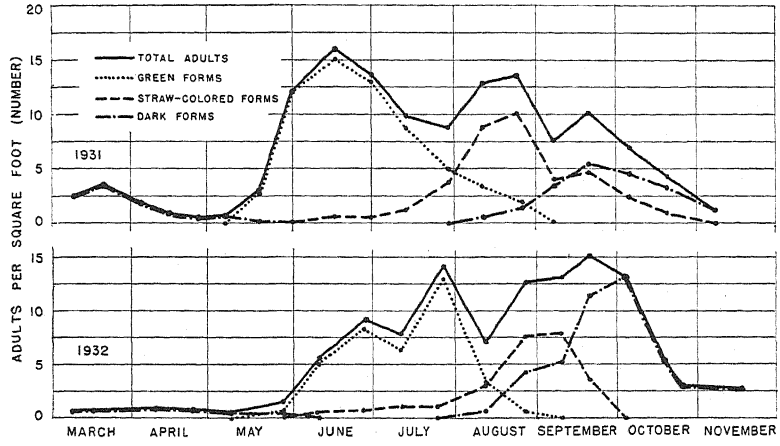


FIGURE 5.—Seasonal coloration of the adult beet leafhopper in the central Columbia breeding area.

the relative egg development for that date. It was later found, however, that almost identical results could be obtained by simply counting the number of mature eggs present in each female on given dates.

The results of these measurements are presented in figure 6. The data indicate a total absence of developing ovules from mid-September until the latter part of February or the first of March of the following year. During April and May the number of developing ovules is much greater than throughout the summer. The decline in May is probably due to exhaustion of the egg supply of the overwintered females and is followed by maturing of spring-brood females.

A peak of ovule development is shown near the end of June 1931 for the spring brood and another the first part of August 1931 for the summer brood. In 1930 a peak is shown for the spring brood the latter part of June. Other peaks for the spring and summer brood do not show in figure 6. They are in all probability masked by overlapping of broods. The abrupt ending of development in September is due

largely to the dying of the summer brood, leaving only the fall females, whose ovaries do not develop until the following spring.

Comparison of figures 6 and 7 indicates that the number of ovules present in the spring females that moved into the beet plots was considerably larger than in those that remained in the breeding grounds.

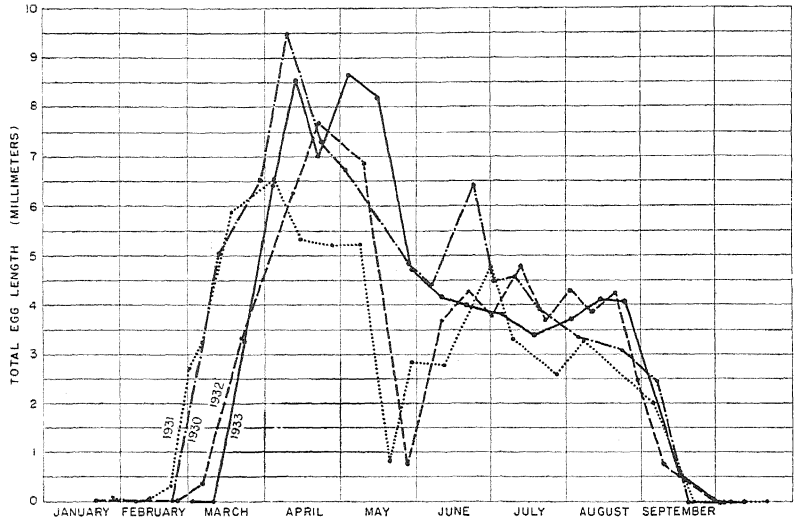


FIGURE 6.—Egg development of the beet leafhopper in the breeding grounds.

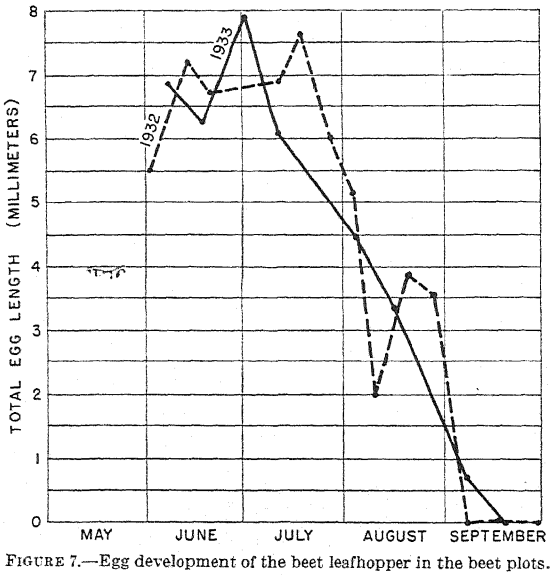


FIGURE 7.—Egg development of the beet leafhopper in the beet plots.

SUMMARY

Ecological studies of the beet leafhopper, *Eutettix tenellus* (Baker), were made in the breeding area in the central Columbia River valley, in south-central Washington and northeastern Oregon. In this area there are three broods of this insect. The more important host plants are tumble-mustard, alfileria, and Russian-thistle.

The leafhopper usually disperses from this breeding area between May 20 and June 30, movements being more or less continuous during this period. Return to the fall and winter hosts, tumblemustard and alfileria, occurs during October and is largely a forced movement caused by the drying of the summer

hosts, principally Russian-thistle. If germination of the fall hosts is delayed by drought until after Russian-thistle has dried, a high mortality is likely to result.

High percentages of curly top are directly correlated with large spring-brood populations of the leafhopper. Large populations of the summer and fall broods are relatively unimportant in this respect, except as they affect the magnitude of the spring brood the following year.

Winter mortality is usually of comparatively little importance in this area. In only one winter of the four observed was there severe mortality, and this was due to subzero weather without snow cover, which not only subjected the insects to intense cold but killed their food plants as well.

Seasonal variations in color patterns of the beet leafhopper have been observed in this area. In general, dark forms predominate during winter, greenish forms during spring, and straw-colored forms during summer.

Studies have been made of seasonal ovule development in this breeding area. Development drops to zero in September and does not begin again until late in February or early in March of the following season.

A TRANSMISSIBLE MOSAIC DISEASE OF CAULIFLOWER [1]

By C. M. TOMPKINS [2]

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INTRODUCTION

A hitherto undescribed mosaic disease of cauliflower (*Brassica oleracea* L. var. *botrytis* L.) was first observed in November 1932, in a field near Alvarado, in the San Francisco Bay section of California. Since cauliflower is a major winter crop in certain coastal valleys in central and southern California, an investigation of the disease seemed desirable. A preliminary report has already been published (*21*).[3] Results of experimental studies on symptomatology, transmission, host range, and identity of the virus are presented in this paper.

REVIEW OF LITERATURE

Within the last 15 years, numerous contributions have been made on virus diseases of cruciferous plants (*1, 2, 3, 4, 5, 6, 7, 8, 10, 12, 13, 14, 16, 18, 19, 20, 23*). These papers, with one exception (*10*) have dealt principally with symptomatology, methods of transmission, and host range, but have presented no data on the properties of the virus or viruses concerned. Hoggan and Johnson (*10*) were the first to report on the properties of a crucifer virus which is transmissible to cabbage, mustard, turnip, and horseradish. In the absence of data, it is doubtful whether the specific identity of other crucifer viruses has been clearly established. When considered as to available facts, all of the crucifer viruses referred to are apparently different from the cauliflower virus reported in this paper.

Only five references to cauliflower mosaic have been found in the literature. Clayton (*3*), in his investigations of "the true cruciferous mosaic", transmitted a virus derived from infected rutabaga plants to healthy cauliflower seedlings. The symptoms consisted of yellow spots scattered over the green leaf, which condition he termed "mosaic." High temperatures (70° to 80° F.) favored symptom expression, while low temperatures (55° to 65°) induced masking. The maximum infection observed in commercial cauliflower plantings was 0.75 percent, and mosaic was stated to be rare. If Clayton's description of symptoms and photographs may be used as criteria for comparative purposes, it is concluded that "the true cruciferous mosaic" is different from the mosaic disease reported in this paper.

[1] Received for publication Nov. 2, 1936; issued August 1937.

[2] The writer gratefully acknowledges his indebtedness to Prof. Ralph E. Smith and Dr. M. W. Gardner for helpful advice and assistance; to Prof. B. A. Madson and W. W. Mackie for providing greenhouse space and facilities; to Elizabeth Wilson for seeds of *Brassica arvensis;* to Dr. R. E. Clausen, for seeds of *Nicotiana langsdorffii;* and to Dr. K. Nakata, Kyushu Imperial University, Fukuoka, Japan, for seeds of *B. pekinensis.*

[3] Reference is made by number (italic) to Literature Cited, p. 45.

Noble et al. (*15*) observed a mosaic disease of cauliflower in New South Wales, Australia, in 1933. In a letter to the writer, Noble [4] stated:

> Our cauliflower mosaic is probably distinct from the Californian type. It is not an important disease here, but infected plants are stunted and show mottling and distortion of the foliage. No necrotic spotting has been observed. The disease is thought to be transmitted by the cabbage aphis, but this has not been studied in detail.

Brief reference to a mosaic disease of cauliflower in Japan was made by Hino (*9*).

In 1935 Smith (*19*) observed a mosaic disease of cultivated crucifers in the Cambridge district of England. He found the virus was readily transmissible to various crucifers, including cauliflower, by means of juice inoculations. Initial symptoms consisted of vein clearing, followed by mottling, distortion, and rosetting. No infection of *Nicotiana glutinosa* L. was obtained. These facts suggest a close similarity between the English virus and the cauliflower virus prevalent in California.

Recently, McWhorter [5] in reporting a mosaic disease of dames violet (*Hesperis matronalis* L.), stated that mosaic diseases of crucifers, especially of cabbage and cauliflower, had been observed in past seasons near Oregon City, Oreg. In October 1935, the writer found a mosaic disease of cauliflower in a commercial planting 5 miles north of Salem, Oreg. The symptoms were typical of the disease which occurs on cauliflower in California.

DISTRIBUTION AND ECONOMIC IMPORTANCE OF THE DISEASE

The mosaic disease of cauliflower is coextensive with cauliflower culture in the coastal valleys of central and southern California. Field observations indicate that all commercial varieties of cauliflower grown in the State are liable to infection. The average incidence of the disease seldom exceeds 30 percent of the plant population. Occasionally in a few fields all plants are infected, but such instances are uncommon. From the producer's standpoint, the principal loss caused by the disease is a marked reduction in the number of marketable heads or curds.

Numerous lots of diseased cauliflower plants, representing most of the important commercial varieties grown in California, were collected at intervals during the seasons of 1933 to 1935, inclusive, in the San Francisco Bay section; in the Salinas, Santa Clara, and Santa Maria Valleys; and in Los Angeles County.[6] One collection was also made inland near Merced in the San Joaquin Valley. Proof of natural infection of field-grown plants was established by successful greenhouse inoculations to healthy young cauliflower plants of the corresponding varieties.

Annual surveys made from July to September 1933 to 1935, inclusive, failed to reveal the disease in any commercial cauliflower seedbeds located in the open field, except as noted in the next paragraph. The prevalence of high summer temperatures and the absence of infective aphids may possibly be correlated with lack of infection.

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[4] Letter dated Sept. 11, 1935, from R. J. Noble, Department of Agriculture, Sydney, New South Wales, Australia.

[5] McWHORTER, F. P. MOTTLING OR BREAKING IN DAME'S ROCKET IN OREGON. U. S. Dept. Agr., Bur. Plant Indus., Plant Disease Reptr. 20: 199. 1936.

[6] Specimens of diseased cauliflower plants were collected near Hawthorne, Los Angeles County, by J. H. Freitag, Division of Entomology and Parasitology, California Agricultural Experiment Station.

In the market-garden areas near Colma, on the San Francisco peninsula, low temperatures prevail throughout the year, permitting continuous cultivation of cruciferous crops, especially cauliflower. Thus a constant source of virus inoculum is available. Infected seedlings are frequently found in seedbeds, usually located at some distance from the cauliflower fields, and the incidence of the disease may vary from a trace to 100 percent.

SYMPTOMS OF THE DISEASE

The sequence of symptom development in cauliflower plants is identical whether the plants are exposed to natural infection in the field or subjected to artificial inoculation in the greenhouse. The first symptom of infection is a clearing of the veins, which usually begins at or near the base of the leaf and gradually extends over the

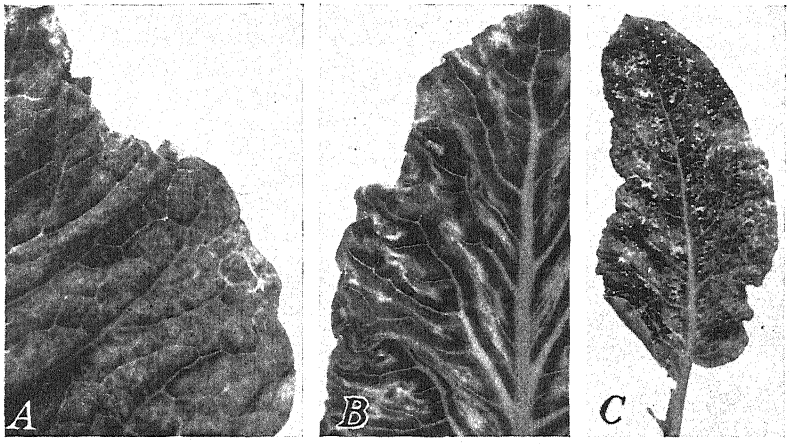


FIGURE 1.—Symptoms produced by the cauliflower mosaic virus on cauliflower leaves under natural conditions: *A*, Vein clearing; *B*, vein banding, mottling, and distortion; *C*, necrotic spotting, curvature of the midrib, and distortion.

entire leaf (fig. 1, *A*; fig. 2). At the onset of the disease, one or several leaves may show vein clearing, but frequently this symptom appears simultaneously on all leaves of the plant.

Vein clearing may persist for 10 to 20 days, but gradually changes to vein banding, which consists of narrow, continuous, dark-green areas parallel with and adjoining the midrib and lateral veins (fig. 1, *B*). The occurrence of numerous, irregularly shaped, nonraised, dark-green islands in the chlorotic areas between the veins results in a mottled effect (fig. 1, *B*).

Later, small, irregular, necrotic lesions appear in the mottled areas (fig. 1, *C*). The lesions, whose light tan color, remains unchanged by age, are different from and cannot be confused with the "ringspot" virus of cabbage and Brussels sprouts recently described by Smith (*19*). A necrotic ring or black-spot virus disease, similar to Smith's ringspot disease but different from cauliflower mosaic, has been under observation and study in the San Francisco Bay section since 1932. The production of necrotic lesions by the cauliflower virus is often accompanied by curvature of the midrib and leaf distortion (fig. 1, *C*).

Early infection usually causes severe stunting of the plants. The terminal heads or curds of stunted plants measure less than 4 inches in diameter, are loosely or not at all covered by small, distorted leaves, and are always slightly discolored and unmarketable. As a rule, late-infected plants are not stunted. Their curds are undesirable for eastern shipment because the mottled leaves often become bleached or yellowed in transit.

Symptom expression in the field depends upon low winter temperatures. Under greenhouse conditions symptoms are well developed on

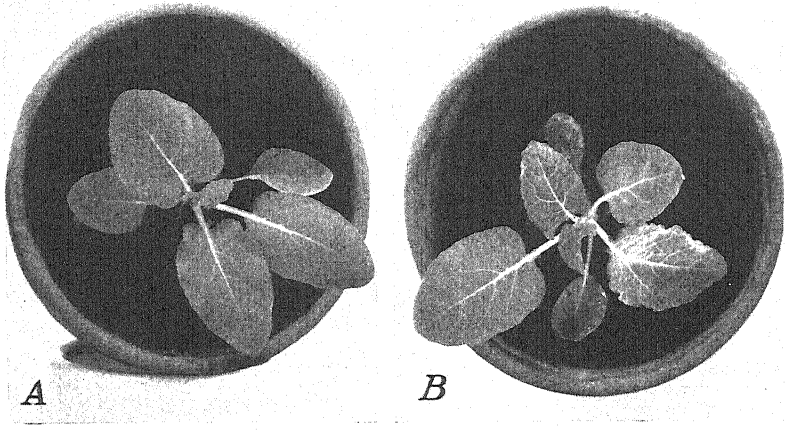


FIGURE 2.—Symptoms produced by the cauliflower mosaic virus on February cauliflower seedlings 15 days after artificial inoculation in the greenhouse at 13° to 19° C.: *A*, Noninoculated control; *B*, infected with the cauliflower mosaic virus.

artificially infected seedlings at temperatures ranging from 10° to 19° C., while higher temperatures favor masking.

MATERIALS AND METHODS

For use in studies on transmission, properties, host range, and varietal susceptibility, a stock culture of the cauliflower mosaic virus was derived in February 1934 from an inner leaf of a mottled and severely stunted cauliflower plant, horticultural variety February, selected near the center of a 10-acre planting at Alvarado. Inoculations were made to healthy February cauliflower seedlings, usually with two to three leaves, and 100-percent infection was obtained. This virus culture was used in all inoculation studies.

Inoculations were made in a greenhouse in which the temperature ranged from 13° to 19° C. The greenhouse was fumigated weekly for the control of aphids and thrips with vapors of nicotine sulphate. For the virus suspensions leaves and petioles from young cauliflower plants showing the first symptoms of the disease were ground in a sterile mortar lined with cheesecloth, and the infective juice was expressed from the macerated pulp. The carborundum method, as devised by Rawlins and Tompkins (*17*), was used.

Aging tests were conducted by pipetting 2 cc of the undiluted inoculum into small, stoppered test tubes which were stored at a constant temperature of 22° C. For determining the inactivation

temperature, 2 cc of undiluted virus extract was pipetted into small, thin-walled, stoppered test tubes, which were then immersed in an electrically heated, agitated water bath for 10 minutes at the desired temperature and cooled rapidly in running tap water. Inoculations were made immediately thereafter. Sterile, distilled water was used as the diluent in preparing the different virus concentrations employed in determining tolerance to dilution.

Since repeated inoculations with the cauliflower mosaic virus to *Nicotiana tabacum* L., *N. glutinosa* L., and *N. langsdorffii* Weinm. failed to produce infection, it was necessary to select a test host in the genus *Brassica*. Because of its marked susceptibility to infection, the February variety of cauliflower was chosen for this purpose. Seeds were planted, as needed, in flats of steam-sterilized soil. Seedlings in the two-leaf stage were transplanted to ordinary soil in 6-inch, porous clay pots. Inoculations were usually made when the seedlings had two or three leaves.

TRANSMISSION

Initial attempts in 1932 to transmit the virus to young cauliflower plants in the greenhouse by means of Holmes' (*11*) rubbing method of inoculation were unsuccessful. Later, infection was readily obtained, as shown in figure 2, by using powdered carborundum (*17*). The incubation period ranged from 10 to 20 days.

Expressed juice from roots of artificially infected February cauliflower seedlings was tested for the presence of the virus by inoculating seedlings of the same variety. Of 42 plants inoculated, 33 became infected, showing that the virus occurs in root tissues and is highly infectious. The incubation period averaged 20 days.

The problem of insect vectors of the cauliflower virus was investigated by Henry H. P. Severin of the Division of Entomology and Parasitology. He found that the following species of aphids which breed on crucifers under natural conditions are vectors of the cauliflower virus: Cabbage aphid (*Brevicoryne brassicae* (L.)), false cabbage or turnip aphid (*Rhopalosiphum pseudobrassicae* (Davis)), and green peach aphid (*Myzus persicae* (Sulzer)), and that 10 additional species of aphids which have not yet been found to breed on cauliflower under natural conditions are capable of transmitting the virus.

In April 1934, flowers produced on 12 mosaic-diseased and 12 healthy cauliflower plants, variety March, were bagged and later hand-pollinated at Alvarado. The seed from both lots was sown in an open field seedbed in August. Later, approximately 2,000 seedlings from each lot were transplanted in an isolated area. Up to harvest time, no infection was observed in either plot, which strongly suggests that the virus is not transmitted through cauliflower seed. Both lots of seed were then tested in the greenhouse under controlled conditions. None of the seedlings in either lot was diseased, substantiating field results.

It is conceivable that the cauliflower virus may be transmitted in nature by agencies other than aphids, as for example, the soil. Investigation into the possible role of such additional agencies was not undertaken.

SUSCEPTIBILITY OF CAULIFLOWER VARIETIES TO ARTIFICIAL INFECTION

All commercial varieties of cauliflower grown in California, as well as some of importance elsewhere, were tested for susceptibility by artificial inoculation in the greenhouse. Seedlings were inoculated after the first pair of true leaves had formed, while a suitable number served as controls. From the results of these tests, shown in table 1, it is apparent that all varieties are highly susceptible. Typical symptoms were shown by all infected plants of each variety tested. Although the incubation periods for varieties grown in California are within the range of 12 to 20 days, a longer period was noted for some varieties which are of commercial importance elsewhere.

TABLE 1.—*Relative susceptibility of certain varieties of cauliflower to the cauliflower mosaic virus as indicated by artificial inoculations into greenhouse-grown seedlings*

Common name or horticultural variety	Plants inoculated	Plants infected		Incubation period
	Number	*Number*	*Percent*	*Days*
April [1]	20	19	95	14-16
Autumn Giant	19	19	100	17-37
Christmas	57	57	100	19-25
Clausdale	15	15	100	27-29
Danish Dry Weather	19	17	89	21-30
Danish Perfection	20	20	100	15
Danish Snowball	20	16	80	26-35
December [1]	20	20	100	14-15
Dryweather Danish Giant	20	20	100	16-20
Early Copenhagen Snowball	20	20	100	20-29
Early Erfurt Snowball	19	17	89	25-31
Early March [1]	60	60	100	15-20
Early Snowball [1]	20	20	100	18
Extra Early Dwarf Erfurt	38	38	100	19-28
Extra Early Paris	20	20	100	21-36
February [1]	160	160	100	14-20
High Altitude Danish Snowball	20	12	60	21-36
Improved Self-Protecting Snowball	54	54	100	22-33
January [1]	20	20	100	14-16
Late March [1]	20	20	100	14
Late Pearl [1]	38	34	89	17-25
Medium Pearl [1]	20	15	75	20-31
Mission Special	20	20	100	20
Model Earliest and Best	15	10	67	21-31
November [1]	20	20	100	14-15
Premium Snow	19	13	68	31-44
Primosnow	18	18	100	26-35
Prizewinner Snowball	10	10	100	24-30
Purple Sicilian Very Early	20	20	100	19-25
Select Early Snowball	20	13	65	25-35
Snowball [1]	35	32	91	20-32
Snow King	20	20	100	25-31
Special Early	20	20	100	14-15
Special Medium	20	20	100	15
St. Valentine	20	20	100	14-15
Super Snowball [1]	20	20	100	17

[1] Varieties of commercial importance in California.

HOST RANGE

NATURAL

In addition to cauliflower, other cultivated cruciferous plants were found to be naturally infected by the cauliflower virus as proved by artificial inoculation with their expressed leaf extract to the test host in the greenhouse. These included kale (*B. oleracea* L. var. *acephala* DC.), Brussels sprouts (*B. oleracea* L. var. *gemmifera* DC.), red and white varieties of cabbage (*B. oleracea* L. var. *capitata* L.), asparagus or sprouting broccoli (*B. oleracea* L. var. *botrytis* L.), and annual stock

(*Matthiola incana* R. Br. var. *annua* Voss). On these hosts, clearing of the veins was the predominant symptom; stunting was not observed.

Virus infection of wild yellow mustard (*B. campestris* L.) and wild radish (*Raphanus sativus* L.) plants is of common occurrence in cul-

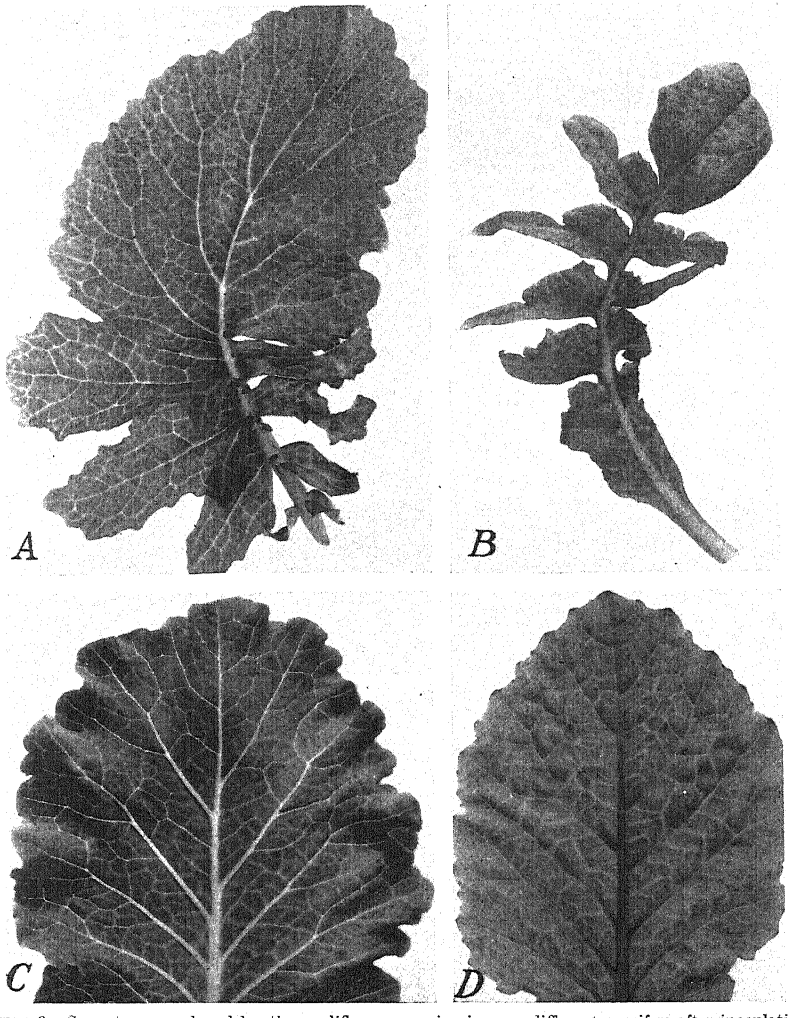


FIGURE 3.—Symptoms produced by the cauliflower mosaic virus on different crucifers after inoculation in the greenhouse at 13° to 19° C.: *A*, Wild yellow mustard; *B*, shepherds-purse; *C*, Winter Colma cabbage; *D*, Mammoth Rock Red cabbage.

tivated crucifer fields or along fence rows and ditchbanks. Generally, infected plants show a pronounced mosaic mottle on the leaves. Failure attended many trials to recover the cauliflower mosaic virus on the test host from such material. However, in January 1935, some wild mustard plants showing symptoms of vein clearing on the leaves were collected at the edge of a field of sprouting broccoli near San Jose, from which the cauliflower virus was readily recovered.

EXPERIMENTAL

Results of artificial inoculation tests in the greenhouse are given in table 2. Vein-clearing symptoms were shown by all plants experimentally infected (figs. 3 and 4). In all cases, the virus was recovered on the test host to establish proof of infection.

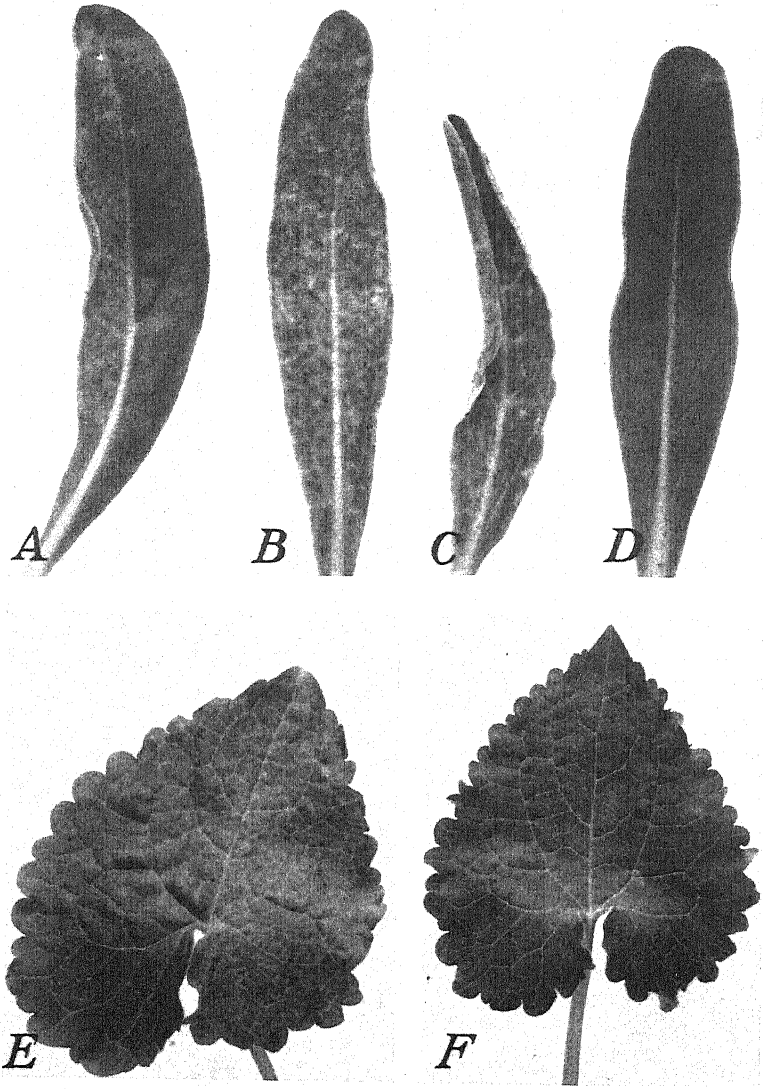


FIGURE 4.—Vein-clearing symptoms produced on leaves of ornamental crucifers by the cauliflower mosaic virus after artificial inoculation in the greenhouse at 13° to 19° C.: *A, B, C,* Infected leaves of annual stock (*Matthiola incana* var. *annua*); *D,* noninoculated control; *E,* infected leaf of honesty (*Lunaria annua*); *F,* noninoculated control.

Infected annual stock (*Matthiola incana* var. *annua*) did not show breaking of the flowers, or petal variegation, a disease recently reported by Tompkins (*22*). Breaking of the flowers of annual stock is

caused by a different crucifer virus, which in turn does not infect cauliflower or cabbage (*22*).

TABLE 2.—*Relative susceptibility of certain cultivated and wild crucifers to the cauliflower mosaic virus as indicated by artificial inoculations into greenhouse-grown seedlings*

Species and common name	Horticultural variety	Plants inoculated	Plants infected		Average incubation period
		Number	*Number*	*Percent*	*Days*
Cultivated hosts:					
Brassica oleracea L. var. *acephala* DC. (kale).	Dwarf Green Curled	20	20	100	15
	Jersey	20	20	100	18
	Siberian or Sprouts	20	20	100	20
	Tall Green Curled Scotch	20	9	45	25
	White Marrow	18	17	94	17
B. oleracea L. var. *gemmifera* DC. (Brussels sprouts).	Long Island Improved	20	18	90	26
	Selected	20	17	85	35
B. oleracea L. var. *capitata* L. (cabbage).	All Seasons	20	11	55	31
	Charleston or Large Wakefield	20	20	100	17
	Copenhagen Market	20	20	100	15
	Danish Ballhead	20	12	60	23
	Early Dwarf Flat Dutch	20	15	75	30
	Early Winningstadt	20	20	100	16
	Glory of Enkhuizen	16	16	100	25
	Hollander	16	15	94	21
	Mammoth Rock Red	20	20	100	21
	Perfection Drumhead Savoy	20	20	100	17
	Round Red Dutch	20	20	100	21
	San Francisco Market	20	20	100	17
	Wisconsin All Seasons	20	12	60	27
	Wisconsin No. 8	20	17	85	27
	Winter Colma	20	20	100	17
B. oleracea L. var. *botrytis* L. (asparagus or sprouting broccoli).	Italian Green Sprouting	20	19	95	18
B. oleracea L. var. *caulorapa* DC. (kohlrabi).	Early Purple Vienna	10	4	40	24
	Early White Vienna	20	20	100	24
B. napus L. (rape)		15	15	100	18
B. campestris L. var. *napobrassica* DC. (rutabaga).	American Purple Top	20	20	100	15
	Harleys Bronze Top	20	20	100	15
	Large White	20	20	100	19
	Monarch or Tankard	20	20	100	13
B. rapa L. (turnip)	Early White Flat Dutch	20	20	100	22
	Purple Top White Globe	20	20	100	18
	Purple Top Yellow Aberdeen	7	7	100	34
	Seven Top	20	14	70	22
	Shogoin or Japanese	18	18	100	19
B. juncea Coss (leaf mustard)	Chinese	20	20	100	14
	Florida Broad Leaved	7	7	100	32
	Fordhook Fancy	20	17	85	17
	Spinach or Tendergreen	8	8	100	27
B. pe-tsai Bailey (pe-tsai)	Chinese pe-tsai	20	18	90	17
	Pe-tsai (Hootooren)	20	20	100	19
	Pe-tsai (Nagasaki Hakusai)	20	20	100	20
	Pe-tsai (Tsuzita Hakusai)	20	20	100	14
B. nigra Koch (black mustard)		10	10	100	29
B. alba Rabenh. (white mustard)		10	7	70	27
Matthiola incana R. Br. var. *annua* Voss (annual stock).	Empress Augusta Victoria	6	6	100	65
	Fiery Blood Red	10	8	80	70
Lunaria annua L. (honesty)		20	20	100	51
Lepidium sativum L. (garden cress).	Curled or Pepper Grass	10	6	60	32
Iberis amara L. (rocket candytuft).		10	3	30	33
Raphanus sativus L. (radish)	Early White Giant Summer	8	4	50	72
	French Breakfast	7	3	43	72
	Long Black Spanish	7	2	29	34
	Long White Japanese	8	4	50	72
	Scarlet Turnip	20	20	100	30
	White Icicle	20	19	95	27
Wild hosts:					
B. adpressa Boiss.		12	11	92	32
B. arvensis (L.) Ktze. (charlock)		25	10	40	61
B. campestris L. (wild yellow mustard).		4	4	100	18
Capsella bursa-pastoris Medic. (shepherds-purse).		10	10	100	29
Raphanus raphanistrum L. (pointed or white charlock).		8	4	50	72

Up to the present time and as far as these studies were carried, no plants other than crucifers have been experimentally infected. Studies on this phase of the problem, to include the testing of a large number of species belonging to other families, are now in progress.

In addition to wild yellow mustard (*B. campestris*) and wild radish (*R. sativa*), infection of other wild crucifers was obtained in the greenhouse, including *B. adpressa* Boiss, *B. arvensis* (L.) Kuntze (charlock), *Capsella bursa-pastoris* (L.) Medic. (shepherds-purse) (fig. 3, *B*), and *R. raphanistrum* L. (jointed or white charlock). It is suggested they may be important as sources of inoculum and as means for overwintering of the virus under natural conditions. This problem is receiving further attention.

The experimental host range, as determined by inoculations, includes 51 vegetable varieties, 3 ornamentals, and 5 wild crucifers.

NEGATIVE REACTIONS

Unsuccessful attempts were made to transmit the cauliflower virus by artificial inoculation to spinach (*Spinacia oleracea* L.) var. Bloomsdale, leaf mustard (*B. juncea* Coss) from Gifu, Japan, rockcress (*Arabis albida* Stev.), wallflower (*Cheiranthus cheiri* L.), blistercress (*Erysimum perofskianum* Fisch. and Mey.), dames violet (*Hesperis matronalis* L.), Virginian stock (*Malcomia maritima* R. Br.), water cress (*Roripa nasturtium* Rusby), golden tuft (*Alyssum saxatile* L.), sweet alyssum (*Alyssum maritimum* Lam.), *Athysanus pusillus* Greene, *Stanleya pinnata* (Pursh.) Britt., *Thysanocarpus radians* Benth., broadbean (*Vicia faba* L.), garden nasturtium (*Tropaeolum majus* L.), celery (*Apium graveolens* L.), tomato (*Lycopersicum esculentum* Mill. var. *vulgare* Bailey), currant tomato (*L. pimpinellifolium* Dunal), bell or sweet pepper (*Capsicum annuum* L. var. *grossum* Sendt.), *Nicotiana tabacum* (var. Turkish and White Burley), *N. glutinosa*, *N. langsdorffii*, head lettuce (*Lactuca sativa* L. var. *capitata* Hort.), and cos lettuce (*L. sativa* var. *romana* Hort.).

PROPERTIES OF THE VIRUS

As shown in table 3, the cauliflower virus retained its infectivity in one trial after storage at a constant temperature of 22° C. for 14 days (336 hours), but was inactivated at the end of 15 days (360 hours). Under conditions of the tests, its longevity in vitro lies between 14 and 15 days. The virus has an inactivation temperature at or near 75° for 10 minutes. A dilution tolerance of approximately 1 to 2,000 was determined for the virus.

DESCRIPTION OF THE CAULIFLOWER MOSAIC VIRUS

Transmitted in nature by *Brevicoryne brassicae* L., *Rhopalosiphum pseudobrassicae* Davis, and *Myzus persicae* Sulzer. Transmissible by artificial inoculation with expressed juice and powdered carborundum. Incubation period 10 to 20 days. Resistance to aging in vitro between 14 and 15 days. Inactivation temperature at or near 75° C. for 10-minute exposure. Tolerance to dilution approximately 1 to 2,000. Cauliflower (*Brassica oleracea* L. var. *botrytis* L.) and certain other vegetable and ornamental crucifers susceptible. On cauliflower, symptoms consist of stunting of plant; vein clearing followed by vein banding, mottling, necrotic spotting, curvature of midrib, and distortion of leaves; dwarfing of terminal head or curd. *Nicotiana tabacum* L., *N. glutinosa* L., and *N. langsdorffii* Weinm. not infected.

TABLE 3.—*Longevity in vitro, inactivation temperature, and tolerance to dilution of the cauliflower mosaic virus*

LONGEVITY IN VITRO, 22° C.

Aged (hours)	Trials	Plants inoculated	Plants infected	Aged (hours)	Trials	Plants inoculated	Plants infected
	Number	*Number*	*Number*		*Number*	*Number*	*Number*
0	5	25	25	168	5	25	25
4	5	25	25	192	5	25	25
8	5	25	25	216	5	25	25
12	5	25	25	240	5	25	20
24	5	25	25	264	5	25	16
48	5	25	25	288	5	25	15
60	5	25	25	312	5	25	6
72	5	25	25	336	5	25	3
96	5	25	25	360	5	25	0
120	5	25	25	384	5	25	0
144	5	25	25				

INACTIVATION TEMPERATURE (10 MINUTES)

Temperature (° C.)	Trials	Plants inoculated	Plants infected	Temperature (° C.)	Trials	Plants inoculated	Plants infected
	Number	*Number*	*Number*		*Number*	*Number*	*Number*
Not heated, used immediately	5	25	25	70	5	25	25
50	5	25	25	75	5	25	0
55	5	25	25	Not heated, used 3 hours after extraction	5	25	25
60	5	25	25				
65	5	25	25				

TOLERANCE TO DILUTION

Dilution	Trials	Plants inoculated	Plants infected	Dilution	Trials	Plants inoculated	Plants infected
	Number	*Number*	*Number*		*Number*	*Number*	*Number*
0	5	25	25	1:500	5	25	25
1:10	5	25	25	1:1,000	5	25	14
1:25	5	25	25	1:2,000	5	25	8
1:50	5	25	25	1:3,000	5	25	0
1:100	5	25	25	1:4,000	5	25	0
1:200	5	25	25				

COMPARISON OF THE CAULIFLOWER MOSAIC VIRUS WITH A TURNIP MOSAIC VIRUS FROM NEW YORK

An earlier suggestion by the writer that the rutabaga virus is different from the cauliflower virus was based entirely upon Clayton's (*3*) description of symptoms and photographs. Comparative inoculation tests could not be made since Clayton's rutabaga virus was not available. However, a mosaic disease of white turnip which causes serious losses in stecklings intended for seed propagation was prevalent in 1934 on Long Island, N. Y., specimens of which were obtained.[7] The virus was readily established on young Purple Top White Globe turnip plants in the greenhouse. Conceivably the turnip virus may be closely related to or even identical with the rutabaga virus (*3*), but experimental evidence is lacking. In a later communication,

[7] Diseased turnips were sent through the courtesy of J. M. Lupton & Son, Inc., Mattituck, Long Island, N. Y.

Clayton [8] stated he had never observed a mosaic disease of turnips on Long Island.

It is interesting to note that after young Winter Colma cabbage and February cauliflower plants had been inoculated with the turnip virus, leaf symptoms developed (fig. 5, *A*, *B*) which were not unlike those induced on Brussels sprouts and cauliflower by the rutabaga virus as depicted by Clayton (*3*) in his figure 1, *B*, *C*, on page 267. The leaf symptoms on cauliflower consisted of slight chlorosis, with small, light-green to yellow circular areas, varying in diameter from 1/16 to 3/16 of an inch, distributed between or adjacent to the veins of the leaf. On cabbage, the lesions were of the same general type, but later the edges became necrotic.

Successful inoculations with the turnip virus were made only to six additional varieties of cauliflower: April, Danish Perfection,

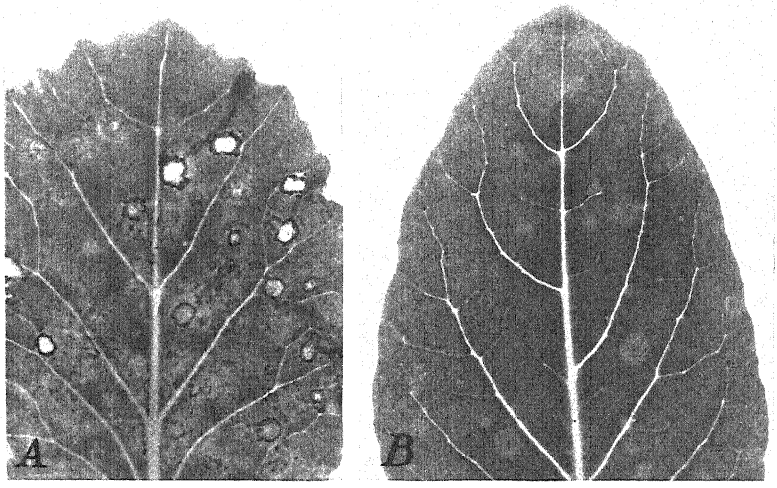


FIGURE 5.—Leaf symptoms produced by a turnip mosaic virus from New York after artificial inoculation in the greenhouse at 13° to 19° C.: *A*, Light-green to yellow circular lesions, becoming necrotic around the edges with age, on Winter Colma cabbage; *B*, pale-green to yellow circular lesions on February cauliflower.

Dry-weather Danish Giant, Early March, Early Snowball, and Super Snowball. The incubation period ranged from 15 to 20 days.

Infection was also obtained on Brussels sprouts, rutabaga, pe-tsai, annual stock (in addition to mottled leaves, the flowers showed breaking), *Nicotiana tabacum* (var. Turkish), and *N. glutinosa*. Asparagus or sprouting broccoli, kohlrabi, radish, and *N. langsdorffii* were not infected.

These limited tests indicate that the turnip virus is different from the cauliflower virus.

SUMMARY

A mosaic disease of cauliflower, caused by a hitherto undescribed virus, is reported in this paper.

The disease occurs chiefly in the cool, coastal valleys of California, frequently causing considerable loss to the crop.

[8] Letter dated Mar. 25, 1936, from E. E. Clayton, Bureau of Plant Industry, U. S. Dept. of Agriculture, Washington, D. C.

Cauliflower mosaic is characterized by stunting of the plant; vein clearing followed by vein banding, mottling, necrotic spotting, curvature of midrib, and distortion of the leaves; and dwarfing of the terminal head or curd.

The insect vectors are *Brevicoryne brassicae*, *Rhopalosiphum pseudobrassicae*, and *Myzus persicae*. The cauliflower virus is also transmissible by artificial juice inoculations when carborundum is used as an abrasive. The virus withstands aging in vitro for 14 to 15 days at 22° C. Its inactivation temperature is approximately 75°, while the tolerance to dilution is about 1 to 2,000.

The host range includes 51 vegetable varieties, 3 ornamentals, and 5 weeds, all belonging to the family Cruciferae.

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RELATION OF CERTAIN DIETARY ESSENTIALS TO FERTILITY IN SHEEP[1]

By GEORGE H. HART, *head, Division of Animal Husbandry*, and ROBERT F. MILLER, *associate animal husbandman, California Agricultural Experiment Station*

INTRODUCTION

Economic studies of the sheep industry show that the percentage lamb crop is the most important single factor in the cost of production. This percentage is usually figured on the number of live lambs born as compared to the number of ewes bred. The number of lambs produced per hundred ewes depends, of course, to some extent on the number of barren ewes and the number producing twins and triplets. Heape[2] gathered statistics on this problem from flockmasters through the Royal Agricultural Society of England. Fertility as recorded by 306 flockmasters ranged from 203.8 to 59.09 percent, and the proportion from 107,603 ewes was 120.4 percent. An analysis of this figure showed that an average of 30 ewes produced twins and 60 ewes produced singles, 90 ewes thus producing 120 lambs; 3 ewes died; and 7 were barren or aborted. The number of lambs produced by 100 ewes was, therefore, 120. Under range conditions in California the usual lamb crop is 80 to 90 percent.

One hundred and twenty-five high-grade yearling Rambouillet ewes were purchased by the University of California in 1927 and 80 Romney-Rambouillet cross-bred ewes were purchased in 1928 for cross-breeding experiments[3] in the production of spring lambs. The Rambouillet ewes came from a long-established flock in which purebred Rambouillet rams had been used for 25 years. The flock, which numbered 6,000 to 8,000 ewes, had ranged throughout the year on the west side of the San Joaquin Valley. It had a lambing record of 80 to 100 percent. The Romney-Rambouillet ewes came from a flock of about 2,000 head which had ranged throughout the year in the Sacramento Valley. The average lamb crop in this flock was about 95 percent. When these ewes came to the experiment station, where feed conditions were better during most of the year, the average production of the Rambouillet ewes rose to 135.5 percent and that of the Romney–Rambouillets to 127.6 percent for the 6 and 4 years respectively that the ewes were in the breeding experiment. For the 3 years in which the ewes were in their prime several lots produced a 160- to 165-percent lamb crop.

These observations suggested the advisability of ascertaining whether any particular dietary essential was necessary for high fertility or whether the problem was merely one of total energy intake.

[1] Received for publication Oct. 29, 1936; issued August 1937. Part of an investigation of the relation of nutrition to reproduction which has been carried on in cooperation with the Bureau of Animal Industry, U. S. Department of Agriculture, since July 1, 1929.

[2] HEAPE, W. ABORTION, BARRENNESS, AND FERTILITY IN SHEEP: AN ABSTRACT OF RECORDS OBTAINED FOR THE YEAR 1896–97. Jour. Roy. Agr. Soc. England (3) 10: [217]–248. 1899.

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EXPERIMENTAL DATA

RELATION OF VITAMIN A TO FERTILITY

Observations on the feeding of breeding ewes had led the writers to believe that vitamin A deficiency might be a factor limiting fertility under practical conditions. An experiment was, therefore, undertaken to determine the influence of vitamin A on fertility. On May 9, 1932, the lambs from 72 of the Romney–Rambouillet ewes used in the cross-breeding experiment mentioned above were weaned and the ewes placed in dry lot on oat hay and a small amount of grain mixture. On May 14 the ewes were segregated into 3 lots of 24 each and fed as follows:

Lot 1. Low vitamin A diet: Oat hay and grain mixture in dry lot.

Lot 2. Low vitamin A diet plus cod-liver oil during breeding: Oat hay and grain mixture in dry lot.

The grain mixture consisted of 40 pounds of dried molasses beet pulp, 20 pounds of cottonseed meal, 20 pounds of rolled barley, and 10 pounds of wheat bran.

Lot 3. Control group maintained on pasture according to usual farm practice. The term "usual farm practice" as used in this paper means that the ewes are maintained in summer on dry native pasture alternated with irrigated Sudan or alfalfa pasture and in winter on native green pasture supplemented with alfalfa hay, silage, or barley.

The experimental animals in these and all subsequent tests had access to brick salt at all times. On June 10, 10 percent of dry skim-milk powder was added to the grain mixture. In the period from June 18 to 22 the oat hay was gradually withdrawn, cottonseed hulls substituted, and the grain mixture increased so that 2 pounds of hulls and one-half pound of grain per ewe were supplied each day.

On August 1, lot 3 was taken from native dry pasture and placed on green Sudan grass during the day. At the same time the administration of cod-liver oil was started in lot 2, each animal receiving daily 10 cc from a pipette directly into the mouth.

On August 6 one ewe was removed from each lot because of poor physical condition. The following day two 2-year-old Hampshire rams were turned in and rotated among the three lots of ewes.

One ewe in lot 2 was accidentally killed on August 20.

On October 7 the breeding period closed, the rams were removed, and the administration of cod-liver oil to lot 2 was stopped. Lots 1 and 2 were both in good condition, but the ewes in lot 3 were somewhat heavier.

During gestation all three lots were handled according to the usual practice on the farm.

During lambing in January the ewes were fed a grain mixture and alfalfa hay. Corn silage was added to this ration for the group that had lambs a week or more old.

On February 1 lambing was about over, the pasture was still short, and the ewes stayed in the corral most of the time. By March 1 native pasture was good and grain feeding was discontinued. The lambs were weaned on April 23. The lambing records of these lots are given in table 1 and the weights of the ewes in table 2.

It will be observed that the ewes lost weight after the lambs were weaned in May. They became quite accustomed to the restricted rations and during the breeding season from August 6 to October 7 the average gain of the three lots was 10.3, 17.1, and 23.4 pounds per head, respectively.

TABLE 1.—*Effect of variations in the vitamin A content of the ration during breeding on the lambing record of ewes, 1933*[1]

Lot no.[1]	Total ewes	Ewes with singles	Ewes with twins	Ewes with triplets	Dry ewes	Total lambs	Lamb crop[2]
	Number	*Number*	*Number*	*Number*	*Number*	*Number*	*Percent*
1	23	19	3	0	1	25	108.7
2	22	14	5	1	2	27	122.7
3	23	9	13	0	1	35	152.2

[1] 1 single lamb died shortly after birth in lot 1. In lot 2 the ewe with triplets died before parturition, 1 pair of twins and 1 single lamb were born dead, and 1 single died shortly after birth. In lot 3, 5 twin lambs, and 2 singles died soon after birth.

[2] Based on the number of lambs alive or dead in relation to the number of ewes bred.

TABLE 2.—*Effect of variations in the vitamin A content of the ration during breeding on the weight of ewes, 1932*

Date (1932)	Lot no.	Ewes	Average weight	Gain or loss per week per ewe	Date (1932)	Lot no.	Ewes	Average weight	Gain or loss per week per ewe
		Number	*Pounds*	*Pounds*			*Number*	*Pounds*	*Pounds*
	1	24	147.9	----------		1	24	135.4	−0.9
May 14	2	24	147.9	----------	July 30	2	24	136.7	+1.3
	3	24	147.9	----------		3	24	146.0	−4.6
	1	24	146.6	−1.3		1	23	139.5	+4.1
May 21	2	24	146.6	−1.3	Aug. 6	2	23	136.1	−.6
	3	24	145.4	−2.5		3	23	137.0	−9.0
	1	24	144.0	−2.6		1	23	139.1	−.4
May 28	2	24	144.0	−2.6	Aug. 13	2	23	137.8	+1.7
	3	24	143.7	−1.7		3	23	139.1	+2.1
	1	24	142.9	−1.1		1	23	144.1	+5.0
June 4	2	24	142.5	−1.5	Aug. 20	2	22	139.8	+2.0
	3	24	143.3	−.4		3	23	140.4	+1.3
	1	24	144.7	+1.8		1	23	143.0	−1.1
June 11	2	24	145.0	+2.5	Aug. 27	2	22	143.6	+3.8
	3	24	145.0	+1.7		3	23	147.2	+6.8
	1	24	137.1	−7.6		1	23	144.3	+1.3
June 18	2	24	137.1	−7.9	Sept. 3	2	22	145.9	+2.3
	3	24	139.2	−5.8		3	23	147.8	+.6
	1	24	140.0	+2.9		1	23	146.3	+2.0
June 25	2	24	140.0	+2.9	Sept. 10	2	22	147.7	+1.8
	3	24	142.1	+2.9		3	23	149.6	+1.8
	1	24	139.8	−.2		1	23	141.7	−4.6
July 2	2	24	140.2	+.2	Sept. 17	2	22	143.8	−3.9
	3	24	140.0	+2.1		3	23	150.9	+1.3
	1	24	139.6	−.2		1	23	144.5	+2.8
July 9	2	24	142.5	+2.3	Sept. 24	2	22	148.4	+4.6
	3	24	----------	----------		3	23	151.3	+.4
	1	24	136.7	−2.9		1	23	149.3	+4.8
July 16	2	24	137.5	−5.0	Oct. 1	2	22	151.3	+2.9
	3	24	----------	----------		3	23	157.6	+6.3
	1	24	136.3	−.4		1	23	149.8	+.5
July 23	2	24	135.4	−2.1	Oct. 7	2	22	153.2	+1.9
	3	24	150.6	----------		3	23	160.4	+2.8

The lamb crop of lot 3, kept under regular farm conditions, was in line with that during the period when they were in the previously mentioned cross-breeding experiments. The restricted diet of lots 1 and 2 had an adverse effect on the lamb crop. Very low vitamin A intake from May 14 to October 7 in lot 1 did not significantly lower the number of lambs as compared to that in lot 2. The addition of the 10 cc of cod-liver oil daily to lot 2 during the breeding period had no significant effect on reproduction, but it may have been a factor in the increased weight gain over the animals in lot 1. The cod-liver oil used in this work when later tested did not prove to be as potent as claimed on the label, but the 10-cc daily doses contained enough of this factor to furnish at least twice the minimum requirement.

RELATION OF VITAMIN A AND PROTEIN TO FERTILITY

In 1933–34, the second year of the study, 7-year-old grade Rambouillet ewes taken from the cross-breeding experiment mentioned above, were put on a diet low in protein and kept on the diet until the close of the breeding season.

The lambs were weaned on April 30, and 80 of the ewes were placed in dry lot on oat-hay and grain mixture.

On June 8 they were divided into four lots, each containing 20 head, and fed as follows:

Lot 1. Low vitamin A diet.
Lot 2. Low vitamin A diet plus cod-liver oil during breeding.
Lot 3. Low vitamin A diet plus Sudan grass pasture during breeding.
Lot 4. Low vitamin A and low protein diet plus cod-liver oil during breeding.

For the first three lots the roughage was a proprietary feed, 2 pounds per head daily, and one-half pound daily per ewe of a grain mixture consisting of 60 pounds of dried molasses beet pulp, 20 pounds of rolled barley, and 20 pounds of wheat bran. The proprietary feed consisted of 60 pounds of cottonseed hulls, 25 pounds of cottonseed meal, and 15 pounds of cane molasses.

Lot 4 received oat hay, 2 pounds daily per ewe, with one-half pound daily of a grain mixture containing 75 pounds of dried molasses beet pulp and 25 pounds of rolled barley.

From July 7 to 16, lot 4 was gradually changed from the oat to hay cottonseed hulls. The total protein intake was thereby reduced from 8 to 5.5 percent. The protein in the hulls is only 20 percent digestible.

One ewe in each of lots 1 and 3 died on July 1. On July 27 a second ewe in lot 1 became sick and was destroyed. Her liver contained 2,500 units of vitamin A.[4]

On August 1 all of the lots began to receive cottonseed hulls, as the supply of the proprietary meal was exhausted. This made it necessary on that date to change the grain mixture for lots 1, 2, and 3 to one made of 50 pounds of dried molasses beet pulp, 30 pounds of cottonseed meal, 10 pounds of rolled barley, and 10 pounds of wheat bran.

On August 19 one ewe from lot 2 was killed for the determination of liver storage of vitamin A, and it was found to contain 4,000 units. This showed that the high vitamin A store in these aged ewes was not being depleted very rapidly even though their diets had been deficient in this substance for 3½ months.

On September 2 the plan of the experiment was therefore changed somewhat. Four ewes from each of lots 1, 2, and 3 and six ewes from lot 4 were eliminated from the experiment. This left 18 head to be kept on low vitamin A rations for determining how long their reserves would carry them. These ewes were killed at intervals of about 2 months and their livers examined. Withdrawal of vitamin A from the liver was found to have been much more rapid during the first year than during the second. The last of the ewes, just maintaining themselves without the drain of reproduction and lactation, lived for over 2 years on the vitamin A deficient diet, and night blindness did not appear until after the lapse of 22 to 23 months.

The four original lots now contained 14 ewes each, except lot 2, which contained 15. Ten cubic centimeters of cod-liver oil per ewe was

[4] This is an antimony trichloride blue unit of vitamin A estimated by the technique outlined by Guilbert and Hart [5] and approximates 0.1 microgram of vitamin A and respresents 0.25 rat unit U. S. P. 1934 revised.

[5] GUILBERT, H. R., and HART, G. H. STORAGE OF VITAMIN A IN CATTLE. Jour. Nutrition 8: 25–44. 1934.

given daily by pipette, beginning September 2, to the animals in lots 2 and 4. This cod-liver oil and that subsequently used contained 2,100 United States Pharmacopoeia 1934 revised units per gram. Tests with the Hilger vitameter A showed it to contain 0.083 percent of vitamin A.

Lot 3 was turned out on barley stubble and alfalfa pasture with some Sudan-grass hay on September 2 for 3 or 4 days, and then on Sudan-grass pasture. On September 10 two Rambouillet rams were turned in and rotated between the groups. By October 12 all the ewes seemed to have been bred. The cottonseed hulls and grain mixture were stopped in all lots and cod-liver oil feeding was stopped in lots 2 and 4.

All the ewes were now fed alfalfa hay and turned out on green feed for about 1 hour a day, the time being gradually increased for a week, after which they were left on the green feed.

On October 26 all lots were turned out with the main farm flock. Alfalfa-hay feeding, one-half pound per head daily, was started on December 20 and on January 2 a grain-mixture feeding was begun.

Lambing began on February 9. The lambing data for these groups are given in table 3 and the weights of the ewes in table 4.

TABLE 3.—*Effect of variations in the vitamin A and protein content of the ration during breeding on the lambing record of ewes, 1934*

Lot no.[1]	Total ewes	Ewes with singles	Ewes with twins	Ewes with triplets	Dry ewes	Total lambs	Lamb crop [2]
	Number	*Number*	*Number*	*Number*	*Number*	*Number*	*Percent*
1	14	5	6	2	1	23	164.3
2	15	6	8	1	0	25	166.6
3	14	3	9	0	2	21	150.0
4	14	6	5	1	2	19	135.7

[1] In lot 1, 2 lambs of 1 set of triplets were born dead and 1 single died shortly after birth. In lot 2, 2 of the triplets and 2 singles were born dead; 1 ewe with prolapsed vagina died and twin lambs were found in her uterus. In lot 3, 1 single lamb was born dead and 1 twin died shortly after birth. In lot 4, 1 single lamb was born dead.

[2] See footnote 2, table 1.

TABLE 4.—*Effect of variations in the vitamin A and protein content of the ration during breeding on the weight of ewes, 1933*

Date (1933)	Lot no.	Ewes	Average weight	Gain or loss per ewe	Date (1933)	Lot no.	Ewes	Average weight	Gain or loss per ewe
		Number	*Pounds*	*Pounds*			*Number*	*Pounds*	*Pounds*
June 8	1	20	141.6		Sept. 30	1	14	148.4	−2.7
	2	20	145.9			2	15	146.1	−2.4
	3	20	147.1			3	14	157.6	+6.2
	4	20	152.0			4	14	148.9	+3.4
June 29	1	18	156.7	+15.1	Oct. 12	1	14	153.7	+5.3
	2	20	154.2	+8.3		2	15	148.1	+2.0
	3	19	154.5	+7.4		3	14	159.0	+1.4
	4	20	156.0	+4.0		4	14	148.6	− .3
Sept. 1	1	18	151.1	−5.6					
	2	19	148.5	−5.7					
	3	18	151.4	−3.1					
	4	20	145.5	−10.5					

The results of this experiment coupled with that of the previous year led to the belief that the animals were not being at all seriously depleted of vitamin A by the methods used. Slaughtered ewes

showed a high vitamin A concentration in the liver. The 5-months period on a low vitamin A diet did not deplete the storage in the old ewes as anticipated. That the vitamin A reserves were not soon reduced was further evidenced by the length of life of the group that was continued on the low intake levels for nearly 2 years before night blindness, the first symptom of vitamin A deficiency, appeared.

The breeding period in 1933 extended from September 10 to October 12, and the average weight change per ewe from September 1 to October 12 in each lot was +2.6, −0.4, +7.6, and +3.1 pounds, respectively, which was not enough according to the general empirical requirements to be considered true flushing. Nevertheless, lot 1 on the vitamin A-low diet with a small gain per ewe produced a 164.3-percent lamb crop. This was not significantly different from that of the ewes of lot 2 which had the same ration as lot 1 plus the cod-liver oil and only maintained their weight during the breeding period. It is therefore apparent that actual gain in weight is not necessary for a high percentage lamb crop provided the ewes are in sufficiently good condition and have enough body stores of the essential food substances.

Lot 3, which was kept on green Sudan grass pasture during breeding and which made the largest gain, did not give birth to as many lambs as lots 1 and 2, but still produced a 150-percent lamb crop, which is very high.

Lot 4 produced a highly satisfactory lamb crop, even though it was the lowest of the four groups and its rations were the most severely restricted.

From the foregoing results it was concluded that the problem would have to be studied with young and growing ewes that had not had the opportunity to store large reserves of the needed dietary essentials in their bodies. Accordingly, it was decided to discard all adult ewes and begin the next year with ewe lambs only.

RELATION OF VITAMIN A TO FERTILITY IN EWE LAMBS

In 1934–35, the third year of the study, vitamin A alone was restricted as in the first year, with the control group maintained under the usual farm conditions. Contrary to the procedure followed in the first year, the low vitamin A diet was maintained throughout the gestation period.

Sixty-one lambs averaging 62 pounds in weight were purchased on June 21, 1934. They were turned on barley stubble until July 24, when they were divided into 2 lots of 30 head each and placed on the following rations:

Lot 1. Low vitamin A diet: Cottonseed hulls and the grain mixture used the previous year after the proprietary meal had been stopped.
Lot 2. Placed on Ladino clover pasture.

The one extra animal was a wether lamb, which was killed July 25. Its liver contained 750 units of vitamin A per gram.

One lamb in lot 2 died on August 29.

On October 22 one lamb from each group was killed and the livers examined for vitamin A. The sample of liver from the lamb of lot 1 contained 1,000 units and that of the lamb from lot 2 contained 1,250 units of vitamin A per gram of tissue, thus showing considerable storage.

For the breeding which started on October 26, a 2-year-old Southdown ram was used. On that date, to facilitate breeding, lot 2 was placed in the corral. This lot was fed the grain mixture and alfalfa hay. On November 1 the animals in lot 1 were thin and not vigorous as compared to the thrifty animals in lot 2.

On December 1 the ram was removed after having been with the ewes for 36 days. Lot 1 was continued on the low vitamin A diet during the gestation period and lot 2 was handled according to the usual practice with the main farm flock. On January 1 the animals in lot 1 were thin as compared to those in lot 2 and on February 1 they still did not appear thrifty.

On February 15 one ewe from each lot was killed for the determination of vitamin A. The animal from lot 1 had 250 units of vitamin A per gram of liver tissue and the animal from lot 2 had 3,300 units.

Of the animals in lot 1, two showed definite and one showed partial night blindness on March 21. Lambing began on that date.

On March 29 one pregnant ewe in lot 1 had a prolapsed uterus. She was killed and her liver was found to contain 25 units of vitamin A per gram of tissue.

On April 8 nine head of lot 1 were night-blind. These were placed in individual pens and fed individually definite amounts of carotene, cod-liver oil, or dehydrated alfalfa meal for a study of the minimum requirements of vitamin A.

On April 20 the remaining 18 ewes in lot 1 were partially or completely night-blind. Nine of these were kept corralled and fed as a group definite quantities of dehydrated alfalfa meal of known carotene content. On April 29 the remaining nine ewes in lot 1 were allowed green feed in addition to a small amount of grain, and on May 7 they were found to have recovered completely normal vision.

The last lamb from the lot 1 ewes was born on April 15 and the last from the lot 2 ewes on April 24.

The lambing data are given in table 5 and the weights of the ewes in table 6.

TABLE 5.—*Effect of vitamin A depletion and of variations in the vitamin A content of the ration during breeding and gestation on the lambing record of ewe lambs, 1935*

Lot no.[1]	Total ewes	Ewes with singles	Ewes with twins	Ewes with triplets	Dry ewes	Total lambs	Lamb crop [2]
	Number	*Number*	*Number*	*Number*	*Number*	*Number*	*Percent*
1	28	8	0	0	20	8	28.6
2	27	13	0	0	14	13	48.1

[1] In lot 1, 7 lambs died shortly after birth. In lot 2, 1 lamb was born dead and 1 died shortly after birth.
[2] See footnote 2, table 1.

TABLE 6.—*Effect of vitamin A depletion and of variations in the vitamin A content of the ration during breeding and gestation on the weight of ewes, 1934–35*

Date	Lot no.	Ewes	Average weight	Gain or loss per ewe	Date	Lot no.	Ewes	Average weight	Gain or loss per ewe
(1934)		*Number*	*Pounds*	*Pounds*	(1935)		*Number*	*Pounds*	*Pounds*
June 21	1	30	62.0	----------	Jan. 2	1	29	75.7	+3.0
	2	30	62.0	----------		2	28	85.6	−3.7
Oct. 1	1	30	67.4	+5.4	Feb. 2	1	29	84.8	+9.1
	2	29	81.5	+19.5		2	28	91.3	+5.7
Nov. 1	1	29	69.0	+1.6	Mar. 2	1	28	87.7	+2.9
	2	28	86.8	+5.3		2	27	96.1	+4.8
Dec. 1	1	29	72.7	+3.7	Apr. 1	1	27	80.2	−7.5
	2	28	89.3	+2.5		2	27	101.1	+5.0

From table 6 it will be observed that from October 1, 26 days before breeding began, and during the 36 days of the breeding period, October 26 to December 1, the ewes of lots 1 and 2 made an average gain of 5.3 and 7.8 pounds, respectively.

The most interesting result of this year's experiment was occasioned by continuing the ewes of lot 1 on a low vitamin A ration during the gestation period. This demonstrated that even young growing ewe lambs that had had access to one season of green feed as nursing lambs stored sufficient vitamin A to enable them to remain on a low vitamin A diet from June 21, 1934, to March 21, 1935, a period of 9 months, before night blindness occurred. When breeding started on October 26 these ewes were not much lower in vitamin A than the lot 2 animals, as determined by liver analysis of one animal from each group. The animals of lot 1 did not do well on the vitamin A-deficient ration and did not have as high a percentage of pregnancies as those of lot 2. Lot 2 was subjected to more favorable conditions than lot 1 and the animals were more thrifty. This probably accounts for the larger lamb crop. Vitamin A cannot be considered the cause of this difference as the data for the next breeding season clearly shows (table 7). No ewe in either lot had twins or triplets. The very significant fact is that in lot 1 the lambs were born weak and all but one died within 5 days while in lot 2, 1 lamb was born dead, 1 died shortly after birth, and the remaining 11 were normal. Since ewe lambs have comparatively few oestrous cycles during the breeding season,[6] more or less difficulty in reproduction and a low percentage lamb crop are to be expected from breeding these animals.

From evidence presented it is clear that vitamin A is highly important in gestation. A high death rate of fetuses in the uterus and a high mortality of living offspring, which are usually born weak, is to be expected if stores of vitamin A become too low in ewes toward the end of gestation.

RELATION OF VITAMIN A, PROTEIN, AND PHOSPHORUS TO FERTILITY

In 1935–36, the fourth year of the study, vitamin A restriction was continued in order to have a group of animals still more severely depleted of this factor at breeding time. In addition, one group of animals was maintained on a low protein and phosphorus ration and one on a low protein ration.

The 54 head that remained from the ewe lambs of the previous year were used. On June 8 they were divided into 3 lots each consisting of 18 head to be handled as follows:

Lot 1. Low vitamin A diet: Cottonseed hulls and grain mixture plus measured quantities of carotene, codliver oil, or dehydrated alfalfa meal to relieve night blindness; 66 pounds of dried molasses beet pulp, 30 pounds of cottonseed meal, and 4 pounds of decarbonized spent bone black.

Lot 2. Low protein and low phosphorus diet: Cottonseed hulls and grain mixture plus cod-liver oil; 96 pounds of dried molasses beet pulp and 4 pounds of oystershell flour.

Lot 3. Low protein diet: Cottonseed hulls and grain mixture plus cod-liver oil; 96 pounds of dried molasses beet pulp and 4 pounds of decarbonized spent bone black.

The 18 animals in lot 1 were the 9 in individual pens and the 9 kept corralled and fed alfalfa meal as a group for minimum requirements.

[6] COLE, H. H., and MILLER, R. F. CHANGES IN THE REPRODUCTIVE ORGANS OF THE EWE WITH SOME DATA BEARING ON THEIR CONTROL. Amer. Jour. Anat. 57: 39–87, illus. 1935.

They were continued through the breeding and gestation periods on close to minimum levels of vitamin A. When their stores of vitamin A were exhausted and night blindness appeared, vitamin A was supplied in measured quantities as carotene, cod-liver oil, or dehydrated alfalfa meal of known carotene content. Four ewes in lot 1 and eight each in lots 2 and 3 had lambed the previous year.

From June 9, when the experimental ration was started, the cod-liver oil was given to lots 2 and 3 twice weekly by adding 125 cc to the concentrates for each lot on Tuesdays and Fridays. Thus the cod-liver oil was administered at the rate of 2 cc per ewe daily and constituted about twice the minimum requirement.

The total protein content of the ration for lots 2 and 3 was 6.8 percent.

On June 19 all lots were on full feed. The animals in lot 2 were beginning to chew the fences.

On July 3 one ewe in lot 1 died of pneumonia.

On July 30 lots 2 and 3 were sheared, the average weight of wool per head being 3 pounds.

On August 16 the ewes in lot 2 appeared thin and were craving something as was evidenced by their eating dirt and wood. On August 20 lot 1 was in good physical condition, but about half of the animals showed some degree of night blindness.

On August 22 breeding began. Two mature Shropshire rams were alternately used between the three lots during the day and at night were turned out on good pasture.

On September 19 three ewes in lot 2 were especially thin and weak. One of them fell down while being driven a short distance to the scales and had to be carried back to the pen after it was weighed. This animal was 3 pounds heavier than at the previous weighing.

On October 23 breeding seemed to be over. Toward the close of the season a Southdown ram had replaced one of the Shropshires In lot 2 three of the ewes had remained too weak to support a ram had they been in oestrum. On October 31 the rams were taken out after a breeding period of 70 days. Cod-liver oil feeding to lots 2 and 3 was stopped on this date, and these two lots were gradually changed to pasture.

On January 3 one ewe in lot 1 died. She had twin lambs in her uterus and her liver contained 3 units of vitamin A per gram. None of the livers of the lambs born in this group showed any trace of color with the antimony trichloride test. The ewes were slaughtered soon after lambing was completed, and an examination of their livers showed from 8 to 80 units of vitamin A per gram.

Lambing began on January 20. The lambing data are given in table 7, the weights of the ewes in table 8, and the phosphorus determinations on the blood of lots 2 and 3 in table 9.

This fourth year of the experiment was the first in which any of the ewes had been so depleted of vitamin A that symptoms of night blindness were manifested at breeding time. Half of the ewes in lot 1 reached this state of depletion and all had very low reserves. The diet of lot 1, except for the deficiency of vitamin A, was better than that of lots 2 and 3 and the animals consumed more of it. Thus lot 1, which showed symptoms of vitamin A deficiency, still gained 13.1 pounds from August 1 to October 31, and had a lambing record of 76.4 percent. All of the lambs, however, were dead at birth or were

very weak and died within 24 hours. Thus the intake of vitamin A in these ewes was sufficient to prevent them from showing any symptoms more serious than night blindness and yet the level was too low for normal gestation.

TABLE 7.—*Effect of vitamin A depletion and of variations in the vitamin A, phosphorus, and protein content of the ration during breeding and gestation on the lambing record of ewes, 1936*

Lot no.[1]	Total ewes	Ewes with singles	Ewes with twins [2]	Dry ewes	Total lambs	Lamb crop [3]
	Number	*Number*	*Number*	*Number*	*Number*	*Percent*
1	17	9	2	6	13	76.4
2	18	7	2	9	11	61.1
3	18	12	3	3	18	100.0

[1] All of the lambs of lot 1 were born dead or died within 24 hours. In lot 2 a single lamb died at birth posterior presentation requiring assistance. In lot 3 all lambs were alive and normal.
[2] No ewes had triplets.
[3] See footnote 2, table 1.

TABLE 8.—*Effect of vitamin A depletion and of variations in the vitamin A, phosphorus, and protein content of the ration during breeding and gestation on the weight of ewes, 1935*

Date (1935)	Lot no.	Ewes	Average weight	Gain or loss per ewe	Date (1935)	Lot no.	Ewes	Average weight	Gain or loss per ewe
		Number	*Pounds*	*Pounds*			*Number*	*Pounds*	*Pounds*
June 8	1	18	99.4		Sept. 19	1	17	110.4	+0.4
	2	18	103.7			2	18	90.2	+3.0
	3	18	105.1			3	18	103.8	+.9
Aug. 1	1	17	104.5	+5.1	Oct. 23	1	17	116.9	+6.5
	2	18	86.0	−17.7		2	18	84.8	−5.4
	3	18	98.7	−6.4		3	18	97.9	−5.9
Aug. 22	1	17	110.0	+5.5	Oct. 31	1	17	117.6	+.7
	2	18	87.2	+1.2		2	18	86.8	+2.0
	3	18	102.9	+4.2		3	18	98.4	+.5

TABLE 9.—*Effect of vitamin A depletion and of variations in the phosphorus content of the ration during breeding and gestation upon the phosphorus content of the blood serum of ewes, 1935*

[Phosphorus in milligrams per 100 cc of blood serum]

Lot no. and number of ewes	Aug. 4	Sept. 13	Oct. 30	Lot no. and number of ewes	Aug. 4	Sept. 13	Oct. 30
Lot 2:	*Milligrams*	*Milligrams*	*Milligrams*	Lot 3:	*Milligrams*	*Milligrams*	*Milligrams*
116	7.84	10.00		110	8.16	6.28	5.84
117	7.69	7.92	6.61	112	6.89	7.73	7.08
119	6.15	6.53	5.09	114	7.48	6.50	
120	7.34	10.52	9.88	151	8.09		7.08
152	4.85	7.73	8.00	153	11.90	16.00	16.00
154	3.94	4.76	5.55	157	8.42		
155	8.25	8.61	7.08	161	9.88	8.42	8.00
160	7.61	7.69	7.62	165	9.09	8.99	10.25
162	6.56	7.44	5.40	166	10.00	9.82	7.62
164	8.90	10.52	9.20	167	8.00	6.99	5.75
172	7.08	6.45	4.39	173	9.64	8.89	
182	6.30	7.62	6.50	175	8.42	8.70	8.16
183	8.00	6.81	7.28	177	7.14	7.24	6.30
184	8.79	6.93		181	8.90	9.20	8.99
189	7.27	6.45	5.06	187	9.52	10.80	8.00
190	6.25	6.96	5.13	191	8.60	9.25	8.42
192	6.25	7.11	7.62	193	7.92	7.18	4.70
199	8.25	8.08	5.67	197	8.00		6.95
Average	7.07	7.67	6.63	Average	8.67	8.80	7.94

Lot 2, on low phosphorus and protein, lost an average of 17.7 pounds from June 8 to August 1 and from then until the end of the breeding period about maintained their weight. This lot had a lambing record of 61.1 percent. All of the lambs were born healthy except one which died at birth. The appetite of lot 2 was noticeably poor, and depraved appetite was observed during breeding. The number of ewes lambing was 9 out of the 18, and 2 sets of twins were produced. Low phosphorus could not definitely be said to be the controlling factor in this year's studies, for cod-liver oil fed as a vitamin A supplement also contained vitamin D and this may have masked the results. Certainly the physical condition of the sheep in this lot was by far the worst of the three groups. Despite this fact table 9 shows no significant lowering of the blood phosphorus by the regimen to which they were subjected. This may probably be explained by the vitamin D in the cod-liver oil drawing phosphorus stores from the bones of the animals and so maintaining blood phosphorus levels at normal

Lot 3 on low protein but ample phosphorus and vitamin A lost 0.3 of a pound per ewe in weight from August 1 to October 31, and still 15 of the 18 ewes lambed. There were three sets of twins and thus a 100-percent lamb crop was produced. All of the lambs were alive and normal.

The data support the assumption that low protein is not as serious in its effect on fertility as low protein and phosphorus. Animals depleted of vitamin A to the point of partial night blindness will still show oestrum, ovulate, and become pregnant. For normal birth of offspring, however, an ample amount of vitamin A must be supplied during the gestation period.

CONCLUSIONS

The data in 1933 showed that low vitamin A intake in adult ewes over a period of 5 months, including the breeding season, did not affect the number of lambs born as compared to control animals supplied with vitamin A above minimum requirements during the breeding period.

The data in 1934 confirmed the findings of the previous year on vitamin A and also showed that limiting the protein intake over a 4-month period including the breeding season had little effect on the number of lambs produced.

The ewes in these experiments were aged and in good condition with large stores of vitamin A in their livers. The high lambing percentage was not accompanied by sufficient gains in weight of the ewes during the breeding season to constitute the empirical requirements for flushing.

When ewe lambs were used and vitamin A intake restricted, they were still not depleted of this food factor at breeding time, but as the low vitamin A diet was continued during gestation depletion of storage occurred after 9 months and seven of the eight lambs born were weak and died soon after birth.

The fourth year the low vitamin A group was depleted at breeding time to the point of night blindness, but still 11 of the 17 ewes conceived and 2 of these had twins. Gestation was unsuccessful, however, and all of the lambs were born dead or died within 24 hours.

The animals on low protein and phosphorus were more seriously affected by the dietary regimen than animals on low protein alone. Depletion of blood phosphorus did not occur on the low protein and phosphorus regimen; the cod-liver oil may have been responsible for this. Further work will have to be done to establish the effect of low phosphorus.

SELENIUM IN PLANTS IN RELATION TO ITS OCCURRENCE IN SOILS[1]

By JOHN T. MILLER, *assistant soil surveyor, Soil Survey Division*, and HORACE G. BYERS, *principal chemist, Soil Chemistry and Physics Research Division, Bureau of Chemistry and Soils, United States Department of Agriculture*

INTRODUCTION

In studying the distribution and intensity of selenium in soils, the writers have made certain observations which it is believed will be of interest to botanists in general and to plant ecologists in particular.

From the studies which followed the recognition of selenium in plants as the cause of a characteristic disease of animals, certain plant relationships have gradually emerged.[2] It first appeared that all plants grown on seleniferous soils absorb selenium to some extent. Later it became apparent that certain plants are particularly prone to this absorption and thereby become intensely toxic (fig. 1). Thus, a sample of *Astragalus bisulcatus* (Hook.) A. Gray from Albany County, Wyo., contained 4,300 parts per million of selenium[2]; a sample of *A. racemosus* Pursh from Dawes County, Nebr., contained 5,560 parts per million; and a sample of *A. pectinatus* (Hook.) Dougl. from Logan County, Kans., contained 1,750 parts per million. A sample of *Stanleya pinnata* (Pursh) Britt. from Otero County, Colo., contained 1,880 parts per million, and a sample of *Applopappus fremonti* A. Gray from Crowley County, Colo., contained 760 parts per million. Such quantities of selenium are lethal when ingested by animals.

On the other hand, many plants on the same areas show no such absorptive capacity. In exactly the same soil area a sample from a plant of *Astragalus bisulcatus* growing among wheat plants contained 1,110 parts per million of selenium while the wheat contained but 45. *A. missouriensis* Nutt. contained but 3 parts per million,[2] where *A. bisulcatus* contained 1,250 parts per million. Native prairie grasses for the most part seem to have a very low absorptive capacity, as illustrated by the fact that on a single square mile in South Dakota where 15 samples of wheat contained an average quantity of 21 parts per million the average for 50 samples of little bluestem was but 0.8. No samples of buffalo grass, of hundreds examined, have been found to contain toxic quantities of selenium.

As pointed out in a recent publication,[3] there appears to be no direct quantitative relationship between the selenium present in a soil and that absorbed by the plants, even of a given species. One reason for this appears to be the variation in the forms of selenium present in soils.[4] Nevertheless, where the soils have a relatively high selenium content, plants in general show increased quantities of selenium.

[1] Received for publication Nov. 27, 1936; issued August 1937.

[2] BYERS, H. G. SELENIUM OCCURRENCE IN CERTAIN SOILS IN THE UNITED STATES WITH A DISCUSSION OF RELATED TOPICS. U. S. Dept. Agr. Tech. Bull. 482, 48 pp., illus. 1935.

[3] BYERS, H. G. SELENIUM OCCURRENCE IN CERTAIN SOILS OF THE UNITED STATES WITH A DISCUSSION OF RELATED TOPICS. SECOND REPORT. U. S. Dept. Agr. Tech. Bull. 530, 79 pp., illus. 1936.

[4] WILLIAMS, K. T., and BYERS, H. G. SELENIUM COMPOUNDS IN SOILS. Indus. and Engin. Chem. 28: 912-914. 1936.



FIGURE 1.—*Astragalus bisulcatus* growing on soil developed from Pierre shale, Fall River County, S. Dak., June 10, 1935.



FIGURE 2.—*Astragalus pectinatus* growing on soil developed from Pierre shale, Shannon County, S. Dak., June 7, 1935.

Even before the recognition of selenium as a cause of the so-called alkali disease an effort was made to relate the incidence of the disease to the soil,[5] and it was shown that the areas best known to produce

[5] FRANKE, K., RICE, T. D., JOHNSON, A. G., and SCHOENING, H. W. REPORT ON A PRELIMINARY FIELD SURVEY OF THE SO-CALLED "ALKALI" DISEASE OF LIVESTOCK. U. S. Dept. Agr. Circ. 320, 10 pp., illus. 1934.

toxic grain were on soils derived from Pierre shales in South Dakota and Nebraska.[5] It was correctly assumed that the source of the injury was in the shales. After the occurrence of selenium in these shales had been established, it was found that selenium was also present in the Niobrara shales and in soils developed from them. These shales, like the Pierre, are a part of the formations of the Upper Cretaceous period. It appears probable that other formations of the Cretaceous period are abnormal with respect to selenium content, and possibly certain younger formations as well.

In the spring of 1935, during the course of a reconnaissance survey of possible seleniferous areas, the writers were impressed by the fact that almost invariably where Niobrara and Pierre formations (fig. 2) were the probable sources of soils, one or more species of *Astragalus* or *Stanleya* were found. In many cases in South Dakota, Nebraska, and Wyoming arrival on these soils was as readily recognized through observation of the botanical associations as through a study of the soils or of the geologic maps. Therefore, during a somewhat detailed study of certain areas in western Kansas, observations were made as outlined below. These observations were further supplemented by observations in Colorado and New Mexico.

OBSERVATIONS IN KANSAS

In Kansas the area of study was confined to the upper drainage basins of the Smoky Hill, Saline, and Solomon Rivers, where these streams cut through the loessial mantle into the Pierre and the soft chalky shales of the upper Niobrara formations (Smoky Hill). The physiography of this region reduced to its simplest terms is that of a plain, slightly tilted toward the east. It is characterized by broad level interstream areas, and short, moderately steep slopes bordering the valleys of streams. Except for restricted areas, the surface of this plain is covered by a thick mantle of loess, which in the northwestern corner of the State attains a thickness of 180 feet. Consequently, the soils over the greater portion of the area are developed from materials of loessial origin. Soils developed from residual materials are confined for the most part to those on slopes bordering streams or in close proximity to streams where the loessial mantle has been removed by erosion, or in first bottoms or terraces where the parent materials are largely residual.

Keith silt loam, a Chestnut soil, is the dominant soil of the uplands. In this soil type the upper 15 inches is dark grayish brown and the upper 3 inches is slightly laminated. At a depth of 15 to 30 inches, the soil consists of a slightly compacted heavy silt loam with a slightly prismatic structure which breaks into clods. This is underlain to depths of 10 feet or more by light-brown silty loess (Sanborn loess). The profile has a heavy accumulation of carbonates at a depth of 20 to 35 inches. The normal vegetative cover where the Keith silt loam is fully developed consists of a dense sod of buffalo grass (*Buchloe dactyloides* (Nutt.) Engelm.) and blue grama (*Bouteloua gracilis* (H. B. K.) Lag.) with lesser amounts of side-oats grama (*B. curtipendula* (Michx.) Torr.). Along with these dominates, occasional plants of a large number of species are scattered over the terrain. These miscellaneous plants increase in number where the range is overgrazed in relation to the grass cover. On this soil type the species

[5] See opposite page for footnote.

of *Astragalus* mentioned above seldom appear even when they are of frequent occurrence on neighboring areas.

At a typical location of Keith silt loam in the SW¼ sec. 7, T. 11 S., R. 28 W., the following cover was observed:[6]

Buffalo and grama grasses, 75 percent.
Miscellaneous vegetation, 25 percent.

The miscellaneous cover consisted of:

Sunflower (*Helianthus annuus* L.).
Russian-thistle (*Salsola pestifer* A. Nels.).
Psoralea (*Psoralea collina* Rydb.).
Sagebrush (*Artemisia Frigida* Willd.).
White wreath aster (*Aster ericoides* L.) (*A. multiflorus* Ait.).
Kuhnia (*Kuhnia glutinosa* Ell.).
Common pigweed (*Amaranthus* sp.).
Blazing-star (*Laciniaria punctata* Hook.).

The surface soil at this point contained but 0.4 part per million of selenium, and the wild aster, which is the best selenium absorbent of this group of plants, contained but 2 parts per million. The other forms of vegetation at this point were not collected for analysis.

Three typical soil profiles, the environments of which differ chiefly in topography, are developed on Smoky Hill shale (upper Niobrara). They are as follows:

(1) On smooth upland: The upper 8 inches consists of dark grayish-brown silt loam. This merges into a brown light silty clay loam which is underlain at 12 inches by a heavy silty clay loam. At a depth of about 35 inches the soil merges into yellowish-brown lighter textured silty clay loam, which in turn merges into the dull-brown Smoky Hill clay shale. The entire depth of this type of profile from surface to shale is 40 to 60 inches. The whole profile is calcareous, with a heavy carbonate accumulation at about 25 to 40 inches.

(2) On moderately steep slopes: The soil profile is similar to that developed on the smooth upland except that both the surface soil and subsoil horizons are shallow and the transitional zone from soil to shale is strongly modified or absent. The unweathered shale occurs within 20 inches of the surface. In places only 1 or 2 inches of grayish-brown topsoil is developed.

(3) On nearly level local alluvial areas: At the base of outcrops of Smoky Hill shale, such as those in the vicinity of the "Pyramids" of Gove County (fig. 3), the soil consists of a yellowish-brown chalky light clay loam which may extend to a depth of 4 feet or more. It is usually undifferentiated, but in places where the accumulation of shale materials is slow, 6 to 8 inches of grayish-brown surface soil has developed.

On all the modifications of the soil developed from the Smoky Hill materials the normal vegetative cover is sparse. In its extreme expression it consists of turpentine weed (*Gutierrezia sarothrae* (Pursh) Britton & Rusby), constituting a 25-percent cover, and a scattering of other plants, making a maximum cover of 35 to 40 percent. In places rabbitbrush (*Chrysothamnus* sp.) is a common plant associated with the turpentine weed. In a few locations *Eriogonum* sp. is common. One of these locations is in the NE¼ sec. 30, T. 13 S.,

[6] Valuable assistance in the identification of plants for this paper was rendered by S. F. Blake of the Bureau of Plant Industry, United States Department of Agriculture, and by E. C. Smith of the Colorado State College of Agriculture, Fort Collins, Col.

R. 35 W., in Logan County. *Mentzelia decapetala* (Pursh) Urban & Gilg., *Aster ericoides* (white wreath aster), *A. fendleri* A. Gray (blue aster), and *Tetraneuris fastigiata* Greene are plants of minor importance on shaly slopes and immediately adjacent areas.

On the soils developed from local alluvium and colluvium adjacent to steep slopes there is a more profuse growth of vegetation. On these areas, however, the vegetative cover is sparse as compared to the normal cover on the Keith silt loam. One of the dominant plants on these soils is turpentine weed, which constitutes from 5 to 25 percent of the cover. At many locations there is a straggly growth of wild aster, interspersed with side-oats grama. In other places one or the other or both of the wild asters mentioned above may constitute a 5- to 10-percent cover. This is especially true on narrow, alluvial

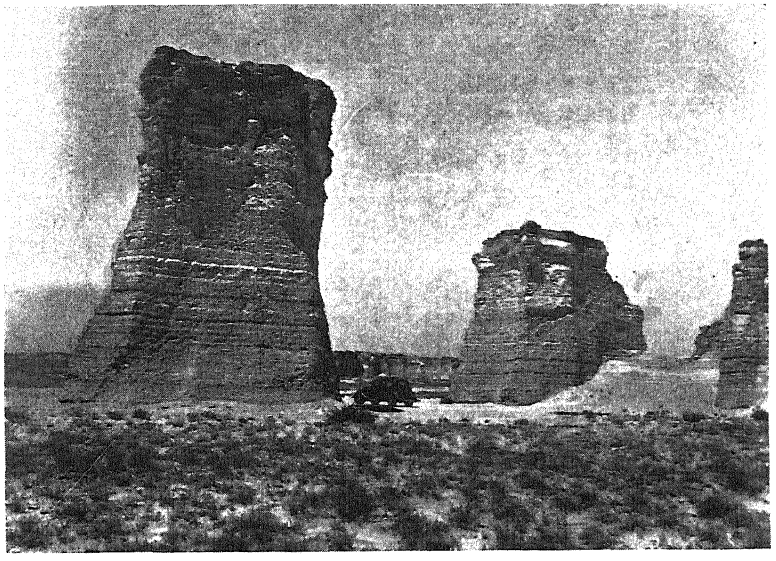


FIGURE 3.—View showing sparse vegetation cover on local alluvium surrounding the "Pyramids," Gove County, Kans. The vegetation in the foreground consists largely of turpentine weed (*Gutierrezia sarothrae*). August 13, 1935.

areas. Little bluestem (*Andropogon scoparius* Michx.), *Muhlenbergia* sp., and *Aristida* sp. are grasses of minor importance. Other plants of minor importance are *Mentzelia decapetala*, *M. nuda* (Pursh) Torr. & Gr., *Psoralea tenuiflora* Pursh, *P. digitata* Nutt., *Lygodesmia juncea* (Pursh) D. Don, Russian-thistle, and sunflower.

At a typical location on a terrace where the soil was derived largely from Smoky Hill materials, an area 50 feet square, 150 feet from the northeast corner of sec. 30, T. 13 S., R. 35 W., in Logan County, was examined in detail. This area was approximately 80 rods from the nearest shale outcrop. The data obtained are as follows: Buffalo grass, 15-percent cover; *Muhlenbergia* sp., 5 percent; *Aristida* sp., 1 percent; a few scattering plants of side-oats grama, 5 plants of *Astragalus pectinatus*, 25 of *Stanleya bipinnata*, Greene, 11 of *Gutierrezia sarothrae*, 2 of *Cirsium lanceolatum* (L.) Hill, 1 of yucca, and 5 of *Helianthus annuus*. There were numerous *Salsola pestifer* plants,

and approximately 1 *Malvastrum coccineum* (Pursh) Gray for each 2 square feet. The total cover was estimated at 40 percent. At this location the surface soil contained 22 parts per million of selenium, the *A. pectinatus* 500 parts per million, based on the air-dry plant, and the *Aristida* 5 parts. The other plants were not examined for selenium.

On another area 50 feet square, located 80 rods south of the one just described, and consisting in part of shaly outcrop and in part of chalky materials of local alluvial and colluvial origin, the cover was as follows: On the shaly outcrop, 20 plants of *Eriogonum* sp. and 1 plant of *Yucca* sp.; on the chalky colluvial material the cover, about 30 percent, consisted of turpentine weed 15 percent, and Russian-thistle 10 percent, along with 20 plants of wreath aster, 5 of blue aster, 10 of *Stanleya pinnata* (Pursh) Britt., and 1 *Psoralea* (*P. digitata*). While these samples were not examined for selenium, the plant association is typical of proven seleniferous soils in this area, and occurring as it does on a shaly outcrop, would justify the assumption that they were seleniferous.

OBSERVATIONS IN COLORADO AND NEW MEXICO

In the spring of 1936 the writers were engaged upon a second scouting expedition through eastern Colorado, New Mexico, Arizona, and Utah, where, on geological and other grounds, the existence of seleniferous areas was suspected. It was observed that wherever the soils were derived from shales corresponding to the Pierre or Niobrara formations, one or more of the plant species which had been found to be good selenium absorbers were always present. A few examples may be cited. In Hamilton County, Kans., no specimens of *Astragalus racemosus* were observed until a small outcrop of soil derived from Niobrara shale, in sec. 2, T. 22 N., R. 43 E., was reached. Here, in a soil sample containing 1.5 parts per million of selenium, and over shale containing 8 parts per million, a plant of *A. racemosus* was growing. It was examined for selenium and found to contain 1,220 parts per million.

In various locations in the Arkansas Valley between Holly and Pueblo, Colo., numerous samples of *Astragalus bisulcatus*, *A. racemosus*, and *A. pectinatus* were collected, and invariably these plants were found to contain selenium in quantities varying from 5 to several thousand parts per million. On United States Route No. 85, from Raton to Las Vegas, N. Mex., wherever outcrops of Pierre and Niobrara shales were found as soil parent materials, various species of *Astragalus* were observed. Five miles from Raton a specimen of *A. racemosus* was collected which contained 390 parts per million of selenium, and the surface soil in which it was growing had 2.5 parts. At a point 14 miles from Raton a sample of *A. bisulcatus* growing on a clay loam which, at the surface, contained 12 parts per million of selenium, was found to contain 2,580 parts. Again, where the route passed over volcanic formations and various other strata, no species of *Astragalus* were observed. Within a few miles of Las Vegas a species of *Astragalus*, tentatively identified as *A. bisulcatus*, appeared. It contained 1,110 parts per million of selenium, and the surface soil in which it was growing contained 1.5 parts. On the highway between Shiprock, N. Mex., and Cortez, Colo., *Stanleya pinnata* (fig. 4) was observed only where the soils were seleniferous. Here samples

were found containing from 70 to 830 parts per million of selenium. In traversing the route from Glenwood Springs, Colo., to Colorado Springs on United States 40 S., no known seleniferous shales outcrop until Walcott is reached. No species of *Astragalus* were noted along this route. North of Walcott the geological map shows a small outcrop of Niobrara shale. A short inspection of this area along State Road No. 11 revealed the presence of numerous specimens of *A. racemosus*. A single plant examined for selenium was found to contain 2,820 parts per million. The upper 6 inches of soil upon which this plant was growing contained 6 parts per million of selenium.



FIGURE 4.—*Stanleya pinnata* plant growing on soil developed from Apishapa shale, Otero County, Colo. August 1, 1936. Note lack of grass cover.

To determine more definitely the relationships indicated by the foregoing data two closely adjacent spots in Kiowa County, Colo., were selected. One of these is a soil area developed over Apishapa [7] (Niobrara) shale and undoubtedly is seleniferous, as indicated in the tabular data. It is located 80 rods north of the southwest corner of sec. 6, T. 19 S., R. 51 W. The other soil is developed over the Ogallala formation (a slightly consolidated sandstone of Tertiary age) and is as free from selenium as can be expected in this area. It is located near the center of sec. 23, T. 18 S., R. 51 W. Both areas are topographically similar and are smooth and uniform in character. At each point an area 100 feet square was selected and the vegetation studied and sampled. At each plot a profile of soil was taken. All the samples obtained were examined for selenium. The results are given in table 1.

The seleniferous area examined was part of a virgin area which probably has never been overgrazed. The cover was estimated: Blue grama, 20 percent; turpentine weed, 10 percent; spurge, 3 percent; miscellaneous plants, 17 percent; total cover, 50 percent. The

[7] In Colorado the upper Niobrara is called Apishapa shale, whereas in Kansas this part of the Niobrara is called Smoky Hill shale.

miscellaneous vegetation included 1 *Astragalus pectinatus*, 10 *Stanleya pinnata*, and 12 *Applopappus fremonti* plants.

The nonseleniferous plot selected was part of an area which had been previously overgrazed. This was selected in order to obtain a variety of vegetation. The normal vegetation of the soil developed on the Ogallala formation consists of a dense cover of grasses, chiefly buffalo and grama grasses. The area selected had an estimated total cover of 75 percent, 50 percent of buffalo and grama grasses, 15 percent of Russian-thistle, and 10 percent of miscellaneous vegetation.

TABLE 1.—*Selenium content of soil profiles and vegetation on a "nonseleniferous" and on a "seleniferous" area* [1]

Area and laboratory sample no.	Field no.	Material	Selenium in—	
			Soil	Vegetation
Nonseleniferous: [2]			*P. p. m.*	*P. p. m.*
B19510	62	Heavy silt loam, 0–6 inches	0.2	
B19511	62	Gritty clay loam, 6–12 inches	.2	
B19512	62	Stiff clay loam, 12–24 inches	.2	
B19513	62	Clay loam, 24–36 inches	.2	
B19514	62	Silt loam, 36–48 inches	.2	
B19515	62	Sandy loam	.2	
B19516	62a	Blue grama (*Bouteloua gracilis*)		0
B19517	62b	Russian-thistle (*Salsola pestifer*)		1
B19518	62c	Scarlet mallow (*Malvastrum coccineum*)		1
B19519	62d	Silky sophora (*Sophora sericea* Nutt.)		1
B19520	62e	Sunflower (*Helianthus annuus*)		1
B19521	62f	Corn [3] (*Zea mays* L.)		1
B19522	62g	Sagebrush (*Artemisia frigida*)		1
B19523	62h	Cocklebur (*Xanthium* sp.)		2
B19524	62i	Spurge (*Euphorbia* sp.)		0
B19525	62j	False buffalo grass (*Munroa squarrosa* Torr.)		1
Seleniferous: [4]				
B19526	63	Gritty clay loam, 0–6 inches	2.0	
B19527	63	Gritty clay loam, 6–12 inches	3.5	
B19528	63	Silty clay loam, 12–24 inches	4.0	
B19529	63	Shaly clay loam, 24–36 inches	4.0	
B19530	63	Apishapa shale, 36–48 inches	3.5	
B19531	63a	Blue grama		2
B19532	63b	Russian-thistle		5
B19533	63c	Scarlet mallow		1
B19534	63d	(*Oreocarya* sp.)		1
B19535	63e	Sunflower		2
B19536	63f	Corn [5]		10
B19537	63g	Stanleya (*Stanleya pinnata*)		330
B19538	63h	Cocklebur		6
B19539	63i	Spurge		10
B19540	63j	False buffalo grass		4
B19541	63k	Turpentine weed (*Gutierrezia sarothrae*)		70
B19542	63l	Applopappus (*Applopappus fremonti*)		320
B19543	63m	Narrow-leaved milkvetch (*Astragalus pectinatus*)		4,000

[1] All analyses by K. T. Williams and H. W. Lakin, Bureau of Chemistry and Soils, U. S. Department of Agriculture.
[2] Fields located in Kiowa County, Colo., near center sec. 23, T. 18 S., R. 51 W.
[3] From cultivated field at edge of plot growing under similar soil conditions.
[4] Fields located in Kiowa County, Colo., 80 rods north of southwest corner sec. 8, T. 19 S., R. 51 W.
[5] Volunteer plant at edge of plot growing under similar soil conditions.

CONCLUSIONS

From observations made over wide areas where seleniferous soils occur in juxtaposition to soils relatively free from selenium, and from the examination of several thousand plant samples for selenium, it is believed that plants may be classified in three broad groups in relation to their tolerance of selenium in the soil. Incidentally, it may be remarked that hydrogen-ion concentration does not appear to be an important influence in relation to selenium absorption. The soils

here specifically mentioned all have a pH value in excess of 7, the range being 7.5 to 8.4.

(1) Plants that absorb selenium readily: Among these are *Astragalus racemosus* (fig. 5). *A. pectinatus*, *A. bisulcatus*, *A. carolinianus* L, *Stanleya pinnata*, *S. bipinnata*, *Applopappus fremonti*, *Xylorrhiza parryi* (A. Gray) Greene, and probably other species of the same and different genera. It is not to be inferred that all species of a given genus possess like properties in this respect, since some of the species of *Astragalus*, notably *A. missouriensis*, *A. mollissimus*, and *A. drummondii* Dougl., have been found to contain very small quantities of selenium even when growing on very seleniferous soil. In almost every location

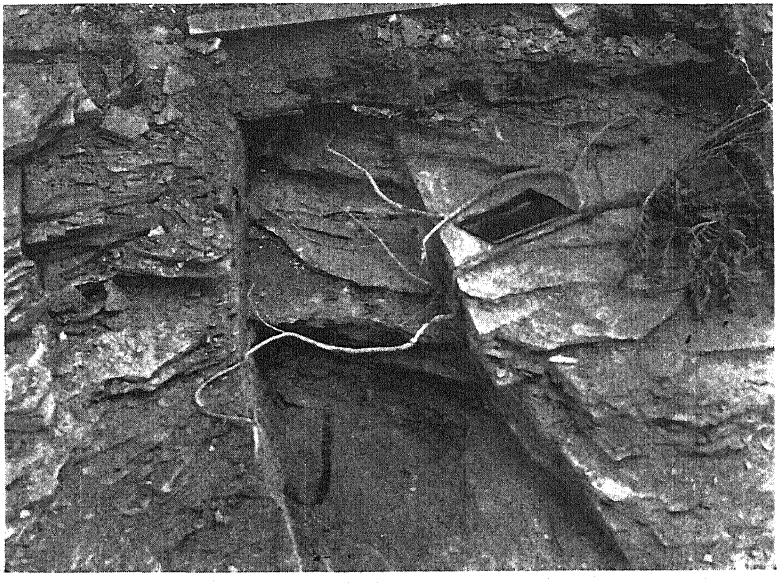


FIGURE 5.—*Astragalus racemosus* root system developed in Apishapa shale. Note limonite and marcasite concretion at location of penknife and small feeder roots of the *Astragalus* at this place in the shale. Pueblo County, Colo., Aug. 29, 1936.

where these highly selenized plants have been found on seleniferous soil, they have been wholly absent or very rare on adjacent nonseleniferous areas. Since analyses show these plants to be consistently high in selenium and to be growing vigorously even with a very high content, it seems possible that selenium may be of importance to their physiological processes. This suggestion is emphasized by the observed fact that in Trego and Ellis Counties, Kans., the three species of *Astragalus* mentioned were found well distributed over the soil areas derived from Smoky Hill shale but were not observed beyond the eastern limit of such soil.

(2) Plants that are able to absorb moderate, or even large, quantities of selenium without severe injury: Such plants are found widely distributed on both seleniferous and nonseleniferous areas. Among such plants are white wreath aster, blue aster, turpentine weed, sunflower, western wheatgrass (*Agropyron smithii* Ryab.), and perhaps others. Included in this group are the common cereals,

wheat, rye, barley, and corn. To what extent the growth of such plants is retarded by the presence of small quantities of selenium is not known, but it is certain that there is a rather low tolerance limit for the soil.

(3) Plants that have a very limited tolerance of selenium and are able to absorb only small quantities when grown on seleniferous soils: In places where the concentration of selenium in the surface soils is high these plants either do not appear or they are of limited occurrence and make poor growth. The most notable examples of this group are buffalo grass and the grama grasses *Bouteloua gracilis* and *B. curtipendula*.

THE SUSCEPTIBILITY OF POTATOES TO THE VEIN-BANDING VIRUS[1]

By LEON K. JONES, *associate plant pathologist*, and C. L. VINCENT, *associate horticulturist, Washington Agricultural Experiment Station*

INTRODUCTION

Tests in connection with tuber indexing of commercial lots of seed potatoes (*Solanum tuberosum* L.) in 1929–32 showed that the vein-banding virus was responsible for a large amount of loss to potato growers in the State of Washington. Since this virus spread rapidly in field plantings in the vicinity of Pullman, an opportunity was afforded to study (1) its rate of spread, (2) its effect upon varieties and seedling clones, and (3) the degree of resistance of varieties and seedling clones to infection under field conditions.

Numerous tests with the vein-banding virus discussed in this paper have shown it to have the following characters: Remains active in vitro at room temperature for 4 to 6 days and in dried plant tissue 17 to 50 days (*1*)[2]; inactivated in vitro at 55° C. for 10 minutes; produces spot necrosis of tobacco and tomato plants when used as inoculum in combination with the potato latent virus; is transmitted by aphids (*Myzus persicae* Sulz.); does not infect *Datura stramonium* L.; and does not modify the expression of tobacco mosaic on tobacco or tomato plants. This virus evidently is a variant of the Y virus (*3*, *10*) and corresponds to potato virus 20 in the tentative classification of viruses by the international committee.[3]

The term latent virus as used in this paper refers to the mild latent form (*6*), a variant of the X virus (*10*) and possibly the same as potato virus 16.[3]

The terms used in the text and in the tables to denote symptoms that were observed in the plants infected by viruses may be defined as follows:

Crinkling.—Slight, wavy unevenness of the leaf blade as contrasted with the smooth appearance of healthy foliage.

Curly dwarf.—Pronounced dwarfing, rugosity, curling, brittleness, and acropetal necrosis of leaves and stems (fig. 1).

Mottling.—Spotting of leaf blades by light green areas which vary in size, shape and degree of paleness.

Necrosis.—Premature death of tissues resulting in a brown discoloration.

Rugose mosaic.—Dwarfing, diffuse mottling, rugosity, necrotic spotting and acropetal dropping of foliage.

Spot necrosis.—Somewhat irregular, necrotic spots and chlorotic lines in the foliage, preceded by vein clearing and rugosity in tobacco leaves, caused by the combined action of the latent and the vein-banding viruses (*6*).

Streak.—Brown to black necrotic areas in leaves and stems of tomato plants, caused by the combination of the potato latent virus and the tobacco mosaic virus (*2*).

Tobacco mosaic.—Mottling of tobacco or tomato foliage caused by the tobacco mosaic virus 1 (*4*).

[1] Received for publication Nov. 30, 1936; issued August 1937. Published as Scientific Paper no. 356, College of Agriculture and Experiment Station, State College of Washington.

[2] Reference is made by number (italic) to Literature Cited, p. 79.

[3] INTERNATIONAL COMMITTEE ON DESCRIPTION AND NOMENCLATURE OF PLANT VIRUSES. ILLUSTRATION OF PROPOSED SYSTEM OF NOMENCLATURE FOR PLANT VIRUSES. 1936. [Mimeographed report.]

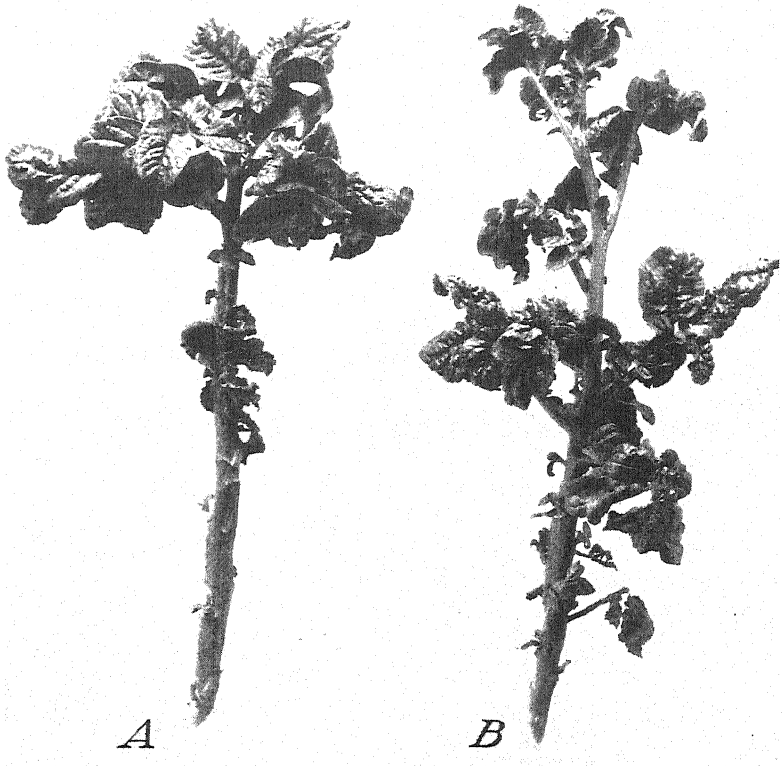


FIGURE 1.—Curly dwarf symptoms produced in potato plants by the vein-banding virus: *A*, Chippewa; *B*, Katahdin.

Top necrosis.—Necrotic spotting and premature death of upper portions of the plant, progressing downward.

Vein banding.—Slight mottling of tobacco leaves by interveinal light green areas in contrast to the normal green adjacent to the veins (*2*).

METHODS

Varieties and seedling clones of potatoes were planted in the field near Pullman each summer from 1932 to 1936, inclusive. At least two field observations were made each season to note the symptoms and the rate of spread of viruses. The viruses occurring in the field plantings have been identified by tests in the greenhouse during the winter months each year. The earlier tests were made with 1 tuber from each hill but in the later tests 5 to 20 tubers from each variety or clone were indexed.

The index potato plants were tested in the greenhouse by inoculating from each potato plant into six tobacco plants as follows: Two with macerated potato-leaf tissue alone; two with macerated potato-leaf tissue plus macerated tobacco tissue infected by the latent virus; and two with macerated potato-leaf tissue plus macerated tobacco tissue infected by the vein-banding virus. Since the latent and vein-banding viruses together produce distinctive symptoms of spot necrosis when incoluated into tobacco plants, the presence of the

vein-banding virus or the latent virus in the potato plants was readily determined.

All inoculations into plants were made with macerated plant tissue according to the method described by Jones (*5*) with the use of carborundum as described by Rawlins and Tompkins (*7*).

FIELD TESTS WITH THE PROGENY OF A VIRUS-FREE EARLY ROSE TUBER

During the winter of 1929, when indexing commercial stock, a single tuber of the Early Rose variety was found to be free from both the latent and the vein-banding viruses (*2*). Virus-free stock from this tuber was developed under cloth chambers in the greenhouse during the winter months of 1929–31 until 142 virus-free tubers were available. This stock was planted in the field in 1932, and its progeny was grown in the field in 1933 and 1934. All plantings were made in

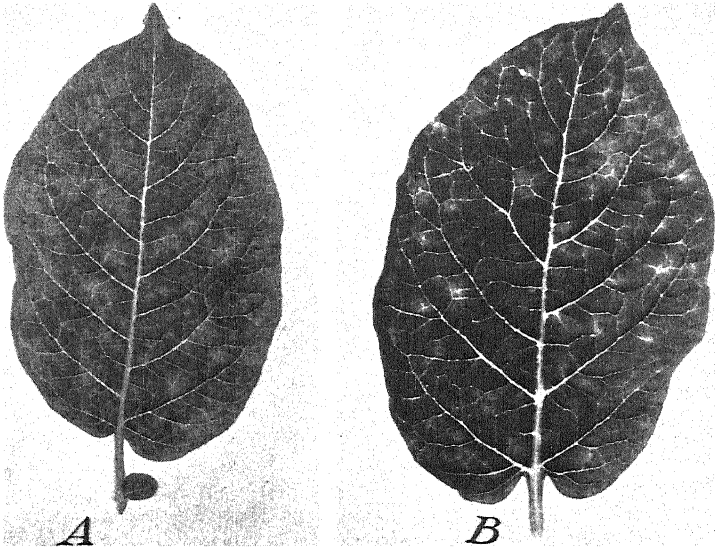


FIGURE 2.—Mottling of potato leaflets caused by the vein-banding virus: *A*, Early Rose; *B*, Katahdin.

locations at least one-half mile from other potatoes, except that virus-free seedlings were included in the planting in 1933.

Each winter one tuber from each hill produced the previous season was indexed in the greenhouse. The nature of the virus found in the plants was studied by inoculations from each potato plant into tobacco plants, according to the method described above.

The results of these tests show that the 142 hills grown in 1932 remained virus-free; the 314 hills grown in 1933 became 59 percent infected; and the 840 hills grown in 1934 became 100 percent infected with the vein-banding virus during the growing season. No other virus was noted in the Early Rose stock. The symptoms produced by the vein-banding virus were similar to those associated with mild mosaic, which included slight dwarfing of the plants and mild mottling and crinkling of the foliage (fig. 2, *A*).

In December 1932, following the harvest of the 142 hills one tuber from each hill was planted in the greenhouse for indexing. After the index tests with tobacco and tomato plants had shown all plants to be free from viruses the plants were grouped in series of four plants each, separated from one another by cloth partitions. The first group of four plants was then inoculated with the latent virus; the second with the vein-banding virus; the third with the combination of these two viruses; and the fourth was uninoculated. Six weeks later inoculations were made from portions of each potato plant into tobacco and tomato plants. A summary of the results of these tests is shown in table 1.

TABLE 1.—*Results of virus inoculations into virus-free Early Rose potato plants,*[1] *Pullman, Wash., 1933*

Inoculum from tobacco plants	Symptoms produced in potato plants	Symptoms produced by inoculations from infected potato foliage in—				
		Tobacco plants			Tomato plants	
		Potato inoculum alone	Potato inoculum plus latent virus	Potato inoculum plus vein-banding virus	Potato inoculum alone	Potato inoculum plus tobacco mosaic virus
Vein-banding	Crinkling, mottling.	Vein banding.	Spot necrosis.	Vein banding.	Vein banding	Tobacco mosaic.
Vein-banding plus latent.	Rugose mosaic.	Spot necrosis.	...do.....	Spot necrosis.	Spot necrosis.	Streak.
Latent	Crinkling.	Mottling	Mottling	...do.....	Mottling	Do.
Control	None	None	...do.....	Veinbanding	None	Tobacco mosaic.

[1] The Early Rose plants were found to be virus-free by inoculations from the foliage of each potato plant into tobacco plants; 4 plants used in each test.

The vein-banding virus, mechanically inoculated into the virus-free Early Rose stock, produced a definite mottling and crinkling of the foliage similar to the symptoms noted in plants naturally infected in the field. The latent virus produced slight crinkling symptoms without mottling, while the combination of vein-banding and latent viruses produced mottling and rugosity of the foliage and necrosis of the lower leaves, symptoms commonly associated with the term "rugose mosaic."

TESTS WITH SEEDLING CLONES

SEED FURNISHED BY THE UNITED STATES DEPARTMENT OF AGRICULTURE

Thirty-one seedling clones, having desirable plant growth, tuber yield, and shape were increased in the greenhouse during the winters of 1931 and 1932 from the seeds of potato (no. x325: Green Mountain × Katahdin) furnished in 1929 by C. F. Clark of the United States Department of Agriculture. Index tests with this stock in the greenhouse during the spring of 1933 showed no viruses present in the tubers. Two to thirteen hills of each virus-free clone were planted in the field along with the virus-free Early Rose stock in 1933. Indexing of one tuber from each hill during the winter of 1933–34 showed that plants of the seedling clones became infected by the vein-banding

virus in the field as follows: 13 clones, 100 percent; 6 clones, over 50 percent; 5 clones, 10 to 50 percent; 7 clones virus-free. The results of these tests indicate that the seedling clones differed in susceptibility to infection by the vein-banding virus.

LOCALLY DEVELOPED SEED

A large number of seedling clones of potatoes have been developed by the junior author, and the more promising ones have been grown on the farm of the State College of Washington for 2 to 13 years. A

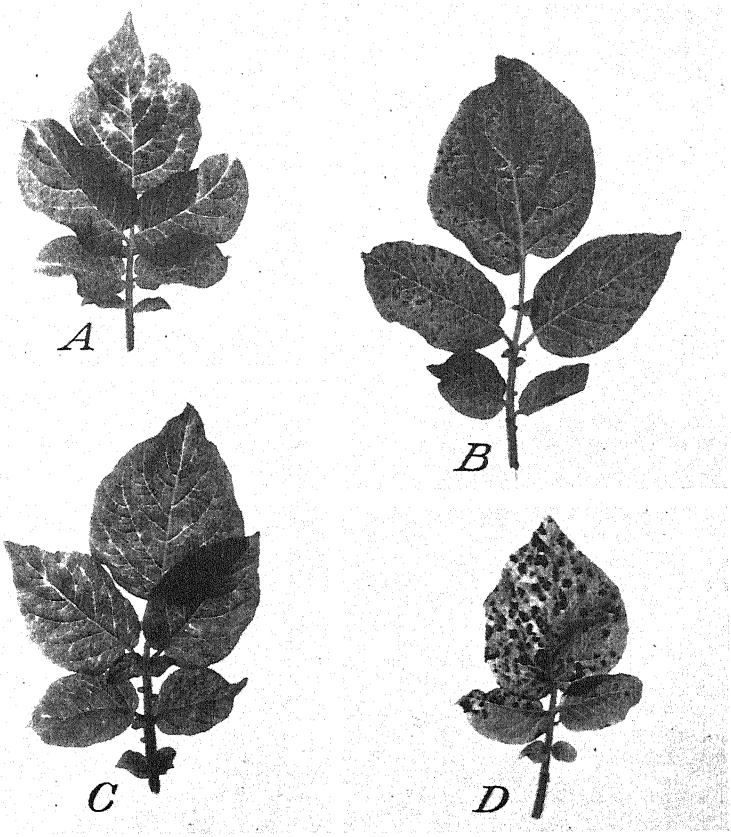


FIGURE 3.—Mottling and necrosis produced in seedling potato leaves by the vein-banding virus: *A*, Seedling 1165–7; *B*, seedling 1152–19; *C*, seedling 1358; *D*, seedling 1045–3.

row of potatoes infected by the vein-banding, latent, and leaf-roll viruses was planted every third to fifth row, throughout the field each season. Although the seedlings were not uniformly exposed to infection by these viruses ample opportunity for infection was afforded; therefore, large and consistent differences in the percent of disease noted in the clones should be significant.

Field examinations of the seedlings were made during 1935–36 and the prevalence of viruses and attendant symptoms was recorded. All of the virus-free clones and those recorded as "virus infection question-

able" from the field observations as well as many of the virus-infected clones were indexed each winter. Five to twenty tubers representing each clone to be tested were planted in the greenhouse and the indexing consisted in observing the symptoms of disease, if any, shown by each plant and inoculating with macerated leaf tissue from each potato plant into tobacco plants according to the method described above.

TABLE 2.—*Results of tests with seedling strains of potatoes to determine susceptibility to infection by the vein-banding virus*

Seedling no.[1]	Parentage	Clones observed	Years grown in field	Clones virus-free[2]	Clones showing symptoms of vein-banding	
					Mottling	Necrosis
		Number	*Number*	*Number*	*Number*	*Number*
50	Early Six Weeks × McCormick	2	12	0	2	
54	Late Rose × Bovee	1	11	0	1	
194–208	Early Northern × McCormick	4	9	0	4	
1028–1064	Keeper, selfed	10	7	0	9	1
1065–1067	Jersey Red Skin × Keeper	10	7	0	10	
1068	Keeper × McCormick	1	7	0	1	
1069	216–3 × 205–2	3	7	0	3	
1145–1148	McCormick × Keeper	8	7	0	7	4
1152–1154	Keeper × 198–13	3	7	0	3	
1157–1169	Irish Cobbler × Keeper	22	6	0	22	
1171–1180	American Wonder × Keeper	22	6	0	20	2
1181	1026, selfed	2	6	0	2	
1203–1358	Russet Burbank, selfed	6	6	0	6	
1425–1426	American Wonder × Keeper	7	6	0	7	2
1582	Irish Cobbler, selfed	1	6	0	1	
1611–1736	do	3	5	0	2	1
1807–1953	Katahdin, selfed	73	4	2	62	11
1954–2082	205–2, selfed	64	4	0	64	1
2103–2150	Irish Cobbler, selfed	3	4	0	3	
2159–2382	194–10, selfed	97	4	0	97	
2384–2417	57–1, selfed	26	4	0	26	
2418–2451	198–2, selfed	21	4	0	21	
2518–2615	Katahdin, selfed	97	2	5	83	9
2516–2630	205–5 × 208–5, selfed	15	2	0	15	
2631–2646	1147–2 × 1046–3, selfed	16	2	0	16	
2647–2745	Katahdin, selfed	99	2	8	75	16
2746–2756	1047–2 × Katahdin	11	2	0	9	2
2757–2842	1321, selfed	86	2	0	86	
2843–2878	1148–1 × 1155–14, selfed	36	2	0	33	3
2879–2902	1157–13 × Katahdin	24	2	1	13	11
2903–2949	1142–1 × 1155–14	47	2	0	43	4
2950	200–5, selfed	1	2	0	1	
2951–2953	1163–1 × 1069–4	3	2	0	3	
2954–2956	Katahdin × Eureka	3	2	0	3	
2957–2973	1148–1 × 1065–3	16	2	0	15	1
2974–3011	Katahdin × 1185	38	2	5	18	15
3012–3026	1159–13 × Katahdin	14	2	0	9	5
3027–3091	205–2, selfed	65	2	0	65	
3092–3099	Katahdin × 1125–9	8	2	0	2	6
3100–3105	Katahdin × 1154–13	6	2	0	4	2
3106–3113	1175–15 × Katahdin	8	2	0	7	1
3114–3127	1148–1 × 1025–2	14	2	0	14	
3128–3133	Katahdin × 1154–3	6	2	0	4	2
3134–3168	200–5 × Katahdin	35	2	1	28	6
3169–3176	Katahdin × 1321	6	2	1	2	3
3177–3182	Katahdin × 1328	6	2	1	3	2
3183–4293	Katahdin, selfed	1,096	2	56	922	118
4294–4326	205–2 × Katahdin	33	2	5	23	5
4327–4375	205–2, selfed	49	2	0	49	
4376–4425	1157–6, selfed	50	2	0	47	3
4426–4509	205–2, selfed	84	2	0	83	1
4510–4542	198–2, selfed	33	2	0	33	
4543–4558	1033–4, selfed	15	2	0	15	
4559–4590	198–2, selfed	32	2	0	31	1
4591–4599	Katahdin, selfed	9	2	4	2	3

[1] Records of seedling clones 50 to 3182, inclusive, were made in 1935. Records of clones 3183–4599, inclusive, were made in 1936.

[2] Determined by field observations on 50 or more hills in each clone, and by greenhouse tests with all clones recorded as virus-free and with many of the clones recorded as virus-infected.

Results of the tests with seedling clones developed from crossed and selfed varieties of potatoes showed that Katahdin was the only parent that transmitted resistance to infection by the vein-banding virus (table 2). Of the 1,572 clones, 89 (5.7 percent) developed from Katahdin as a parent remained virus-free in the field for 2 to 4 years. The remaining 878 clones developed without Katahdin as a parent became 100-percent infected by the vein-banding virus during the 2 to 12 seasons that they were grown in the field.

Marked variation in symptoms, from little or no mottling to moderate or strong mottling of the foliage as well as various degrees of stem

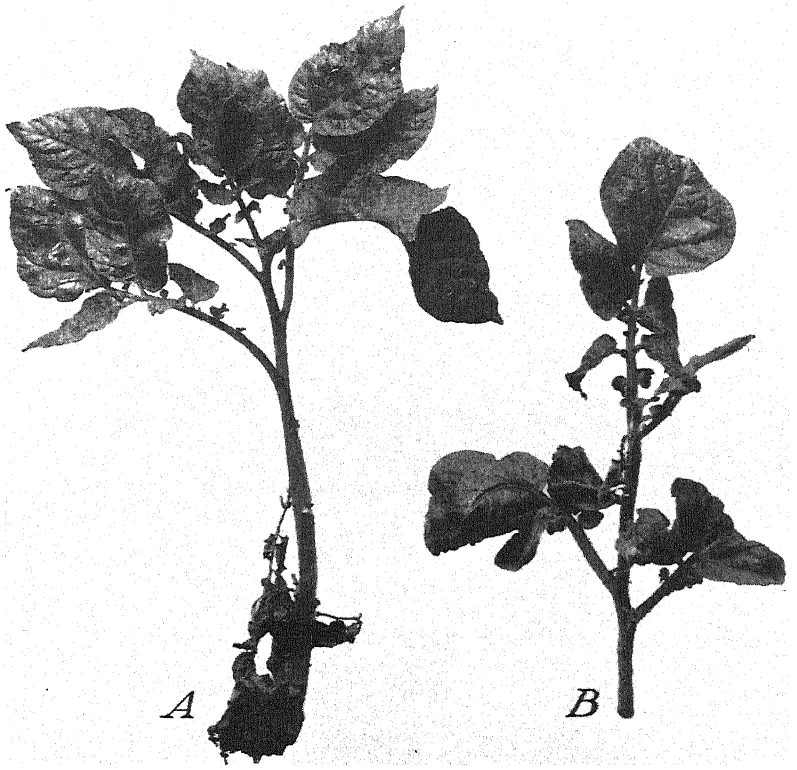


FIGURE 4.—Variation in symptoms produced in seedling potatoes by the vein-banding virus: *A*, Acropetal necrosis in seedling 1425-9; *B*, acronecrosis in seedling 1149-2.

and leaf necrosis, was noted in plants infected by the vein-banding virus only (figs. 3 and 4). These observations are similar to those reported by Schultz et al. (*9*). This variation in symptoms was exhibited by different clones, as the symptoms in all the infected plants of a given clone were uniform. The inoculations into tobacco plants from macerated foliage from each potato plant showed that no X-type virus was associated with this variation. Although some unknown virus that could not be detected by mechanical inoculation into indicator plants of tobacco may have been present, it appears that the variation in symptoms in the different clones was caused by the vein-

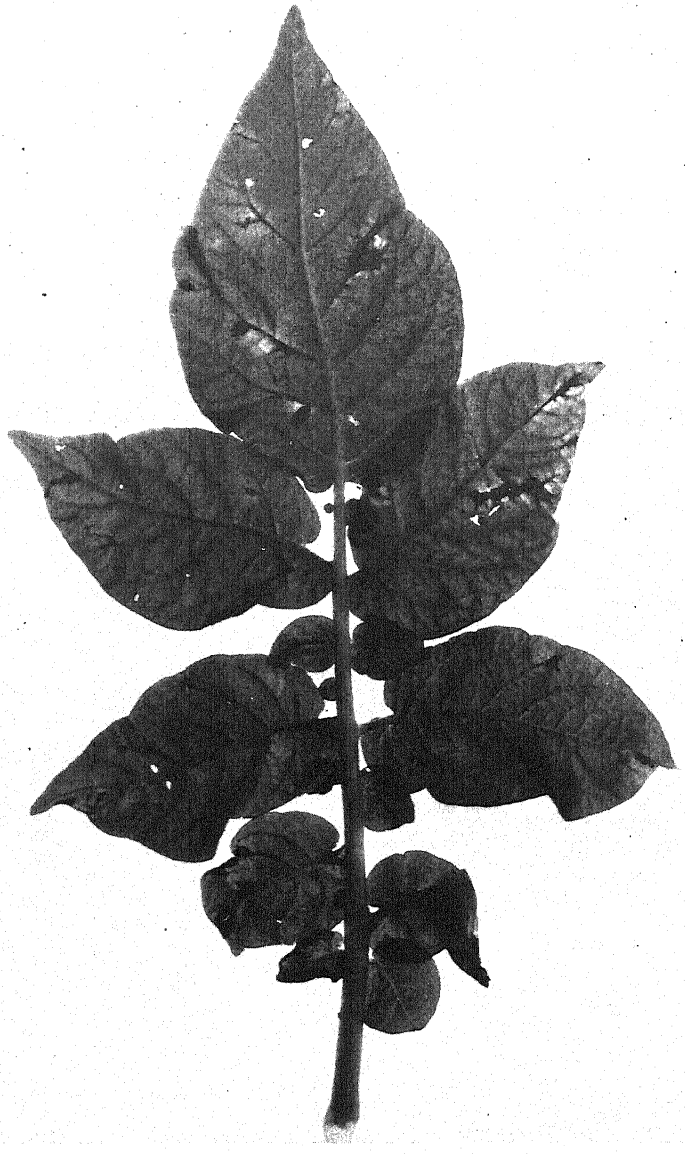


FIGURE 5.—Necrosis in veins in leaf of Chippewa, a symptom of current season infection by the vein-banding virus.

banding virus alone. Curly dwarf symptoms with acropetal necrosis and necrosis in leaf veins (figs. 1 and 5), described in England (*10*) as symptoms associated with Y-virus infection, were noted in many of the clones.

In many of the clones all the plants have shown only a mild to moderate mottling in the foliage and have continued to grow vigorously and produce a good yield in the field even though infected by the vein-

banding virus. This indicates the possibility of producing seedlings that will be highly tolerant to the presence of this virus.

Clones 1028–9, 1066–3, 1145–19, and 1358 showed low percentages of infection by the latent virus, and clones 1069–9, 1154–12 and 1167–7 showed low percentages of infection by the leaf-roll virus, during the 6 to 12 years of field culture. No other viruses appeared in the field plantings.

TESTS WITH COMMERCIAL VARIETIES

Seven commercial varieties of potatoes, and strain no. 41956 furnished by the United States Department of Agriculture, were grown in 1934 and 1935 in the field with the seedling clones to determine the rate of spread of the vein-banding virus to these varieties. Field observations during the early part of the first growing season showed that the Bliss Triumph, Gold Coin, Irish Cobbler, and Russet Burbank were practically free from rugose mosaic and other viruses. Tests in the greenhouse showed that the above four varieties were carriers of the latent virus, while the Chippewa, Katahdin, Warba, and U. S. 41956 were free from both the latent virus and the vein-banding virus when planted in the field in 1934.

Observations were made each growing season to determine the rate of spread of viruses, based on symptom expression. Following harvest in 1935 representative samples of 10 to 40 tubers from each variety were indexed and tested on tobacco plants to determine their virus content (table 3).

TABLE 3.—*The spread of the vein-banding virus to varieties of potatoes grown in the field for 2 years, Pullman, Wash., 1934–35* [1]

Variety	Hills harvested in 1935	Symptoms	Hills affected with vein banding
	Number		*Percent*
Bliss Triumph	125	Rugose mosaic	[2] 100
Chippewa	1,083	Curly dwarf	40
Gold Coin	95	Rugose mosaic	[2] 100
Irish Cobbler	857	do	[2] 100
Katahdin	2,777	Curly dwarf	31
Russet Burbank (strain 1)	1,124	Rugose mosaic	[2] 70
Russet Burbank (strain 2)	778	do	[2] 25
U. S. 41956	33	Mottling	100
Warba	1,083	Curly dwarf	100

[1] Greenhouse tests showed that the Bliss Triumph, Gold Coin, Irish Cobbler, and Russet Burbank stock was 100-percent infected by the latent virus, and that the Chippewa, Katahdin, U. S. 41956, and Warba stock was virus-free, when planted in the field in 1934.

[2] Mixed infection by the vein-banding and latent viruses.

These tests showed that all the varieties are susceptible to field infection by the vein-banding virus. The Katahdin, Chippewa, and Russet Burbank varieties showed some resistance by not becoming 100-percent affected during the 2 years in the field; all other varieties became 100-percent affected under similar conditions of exposure. The Katahdin, Chippewa, and Warba varieties when infected by the vein-banding virus usually show a definite mottling of the foliage (fig. 2, *B*) and necrosis in the veins of the lower leaves (fig. 5) during the season of infection, but the progeny ordinarily shows extreme curly dwarf symptoms in the next season (fig. 1). Of the three varieties, Warba was the most susceptible to infection and showed the most

severe curly dwarf symptoms. Seedling U. S. 41956, which is resistant to the latent virus (*3, 8, 9*), is very susceptible to infection by the vein-banding virus, but the infected plants show only a moderate mosaic mottling without necrosis.

Gold Coin, Bliss Triumph, Irish Cobbler, and Russet Burbank plants, which are carriers of the latent virus, show mottling and acropetal necrosis (rugose mosaic) when infected by the vein-banding virus.

Series of 5 to 50 potato plants were inoculated with the vein-banding virus in the greenhouse during the winter months of 1934–36 as follows: six series of Katahdin (totaling 129 plants); two series of Green Mountain (18 plants); two series of Irish Cobbler (10 plants); and one series of Early Rose (10 plants). With each series of inoculated plants a like number of potato plants were left uninoculated as controls. Several sources of the vein-banding virus were used in the inoculation of the different series as follows: Early Rose, Katahdin, seedling 2038, and seedling 2277. The viruses were obtained from potato plants naturally infected in the field and were transferred to tobacco plants in the greenhouse before being inoculated into the potato plants. The four isolations of the vein-banding virus were inoculated into different series of Katahdin but the viruses from Early Rose and seedling 2038 were used in the inoculations into the other varieties.

The potato plants inoculated with the vein-banding virus became infected as follows: Katahdin, 84 percent; Green Mountain, 84 percent; Irish Cobbler and Early Rose, 100 percent. The lower percentage of infection of the inoculated Katahdin and Green Mountain plants indicates a degree of resistance to the vein-banding virus.

Virus-free Katahdin, mechanically inoculated with the vein-banding virus, showed necrosis and curly dwarf symptons, whereas latent-virus-infected Green Mountain, Irish Cobbler, and Early Rose, mechanically inoculated with the vein-banding virus, showed rugose mosaic symptoms. The symptoms produced by mechanical inoculation were similar to those observed in plants naturally infected in the field.

SUMMARY

Field and greenhouse tests with progeny of a single virus-free Early Rose tuber and seedling clones of potatoes have shown that the vein-banding virus spreads very rapidly in the vicinity of Pullman, Wash.

Tests with seedlings developed from crossing and selfing American Wonder, Bovee, Russet Burbank, Early Northern, Early Six Weeks, Irish Cobbler, Jersey Red Skin, Katahdin, Keeper, Late Rose, and McCormick varieties and their seedling progeny have shown that only the Katahdin variety transmitted to progeny resistance to infection by the vein-banding virus.

In tests with the seedling clones marked variation in symptoms, from little or no mottling to moderate or strong mottling of the foliage, as well as various degrees of stem and leaf necrosis, was noted in plants infected by only the vein-banding virus. This variation in symptoms was exhibited by different clones, as the symptoms in all of the infected plants of a given clone were uniform.

Chippewa, Green Mountain, Katahdin, and Russet Burbank showed some resistance to infection by the vein banding virus as

compared with the very susceptible Bliss Triumph, Early Rose, Gold Coin, Irish Cobbler, and Warba.

Chippewa, Katahdin, and Warba, which were virus-free, usually showed dwarfing and extreme necrosis symptoms when infected by the vein-banding virus. Bliss Triumph, Early Rose, Gold Coin, Green Mountain, Irish Cobbler, and Russet Burbank, which were carriers of the latent virus, showed rugose mosaic symptoms when infected by the vein-banding virus.

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OBSERVATIONS ON THE EFFECT OF ENVIRONMENTAL CONDITIONS ON THE STRUCTURE OF THE LATERAL ROOTS IN SUGAR BEET [1]

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INTRODUCTION

Plants of sugar beet (*Beta vulgaris* L.) at State College, N. Mex., which were being grown for seed production by the overwintering-in-the-field method in the fall of 1935 and spring of 1936, afforded an opportunity for the study of growth and development under a variety of conditions. In the experimental plantings [2] the influence of previous cropping practices upon seed yields was being determined by a series of replicated plots. In this series of plots during the spring and summer of 1935 such crops as cantaloups (*Cucumis melo* L.), cowpeas (*Vigna sinensis* (Torner) Savi), *Sesbania* spp., corn (*Zea mays* L.), and Sudan grass (*Sorghum vulgare* var. *sudanense* (Piper) Hitchc.) had been grown, and the effects of these crops on the subsequent sugar-beet seed crop, in contrast to fallowing the soil, were under test. In the fall of 1935 noticeable differences in the top growth and apparent vigor of the sugar-beet plants were evident, the fallow plots showing greatest growth; the preceding crops in their effects could be ranked in the order given, *Sesbania*, corn, and Sudan grass being definitely retarding in their influence on fall growth. The inequality found in the fall was apparent even after growth started in 1936. However, about the middle of March the different plots began to take on a more uniform appearance. With the advent of seedstalk formation, plots that had made a relatively poor showing in the fall, such as those previously cropped to corn, could hardly be distinguished from the better looking plots of the series.

The variations in soil type, field contour, insect infestation, and previous handling made it possible to secure from the experimental plots a number of collections of sugar-beet roots representing a range of exposures to diverse environmental conditions. Attempt was made by study of lateral root structure to link the deviations from normal development with the environmental conditions which obtained in the soil surrounding the roots selected. The study was limited to the lateral root structures and is presented as a preliminary approach in a field of research about which there exists little information.

MATERIAL AND METHODS

The material for study was obtained from the overwintered sugar-beet plants, after active growth had been resumed in the spring. The

[1] Received for publication Oct. 29, 1936; issued August 1937.

[2] Cooperative experiments on sugar-beet seed production of the New Mexico Agricultural Experiment Station and the Division of Sugar Plant Investigations, Bureau of Plant Industry, U. S. Department of Agriculture.

sugar-beet seed had been planted in early September; development of the seedlings was rapid during the fall and early winter. Growth continued even during the brief winter months and was actively resumed early in March. The plants to be examined were dug with a large clump of the adobe soil adhering and taken to the laboratory. The plants with the adhering soil were soaked in a large tub filled with water. The loosened soil was then carefully removed, after which each taproot with the laterals was placed in a shallow dish of water. The rootlets were cut off close to the taproot and tied together in bundles of about 100 each. The bundles were then cut to a length of three-quarters of an inch, fixed, embedded, and cut as single units. This arrangement made possible the handling of a large number of rootlets and facilitated comparative study under the microscope. In one or two cases where the stand was sparse only two taproots were used as a source of lateral rootlets, but in most cases five or six taproots furnished the lateral rootlets used in this study. The material was fixed in Karpechenko solution and stained with Delafield haematoxylin.

DISTRIBUTION, ORIGIN, AND STRUCTURE OF LATERAL ROOTLETS

The lateral rootlets of the sugar beet are filiform and generally are spread horizontally, remaining confined mostly to the topsoil to the depth of a deep furrow. According to Andrews,[3] the lateral roots permeate every cubic centimeter of topsoil to a depth of 8 to 14 inches and extend laterally 2 feet or more. The adobe nature of the soil made it difficult to trace the individual rootlets for more than 15 inches, but the observations by Andrews cited above and those of European investigators[4] show conclusively that the horizontal spread of the laterals extends some distance beyond the confines of the individual rows.

The lateral roots are whitish and, for any given taproot, of approximately equal diameter, save for a few scattered individuals. They occur in two more or less distinct rows along the flattened side of the taproot. As is usual for lateral rootlets, those of the beet have their origin in the pericycle (pl. 1, *A*); but, as the taproot increases in thickness, laterals that are later formed arise from the more peripheral rings of growth. The rootlets are covered for a considerable distance with rather long root hairs that remain alive for a long time.

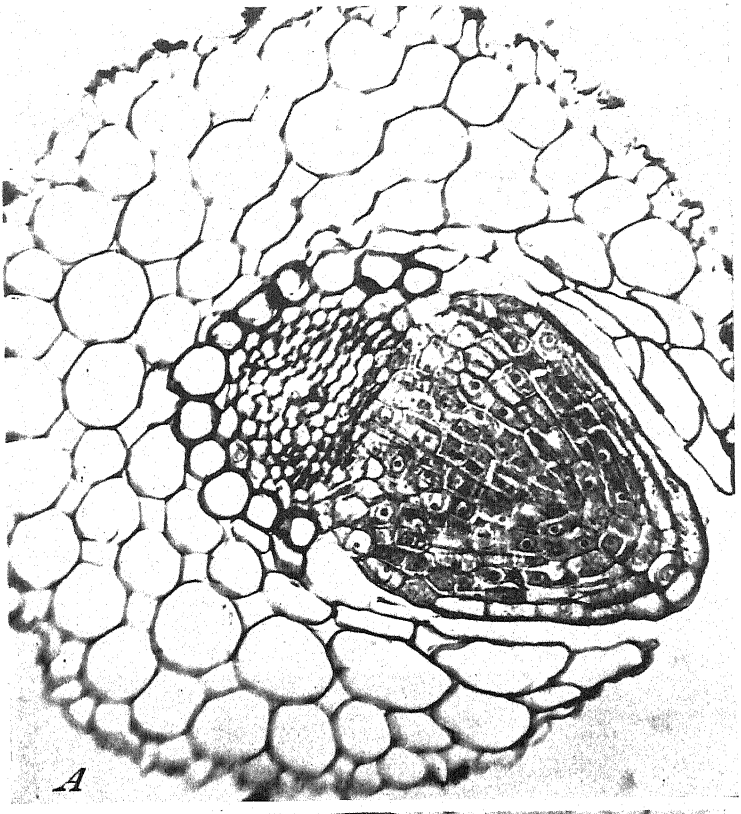
The anatomical structure of a lateral rootlet is simple; each rootlet consists of a broad though few-celled cortex surrounding a miniature stele with the usual type of tissue arrangement (fig. 1). This structure differs from that of the taproot in its protoxylem plate, in the absence of secondary growth, and in the lack of periderm.

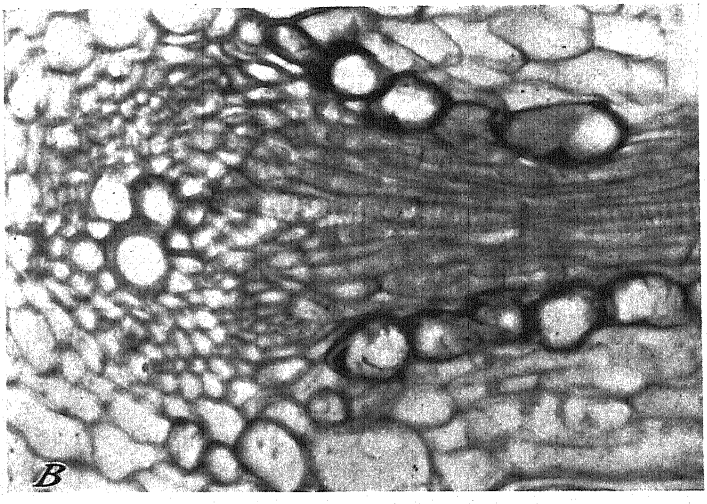
CENTRAL CYLINDER WITH ITS ENDODERMIS

The central cylinder is made up of the protoxylem plate with alternating phloem groups, a single-layered pericycle, and a band of parenchyma between xylem and phloem, in which a few tangential divisions, the initials of a rudimentary cambium, are discernible. Similar tangential divisions may be observed in the pericycle, and

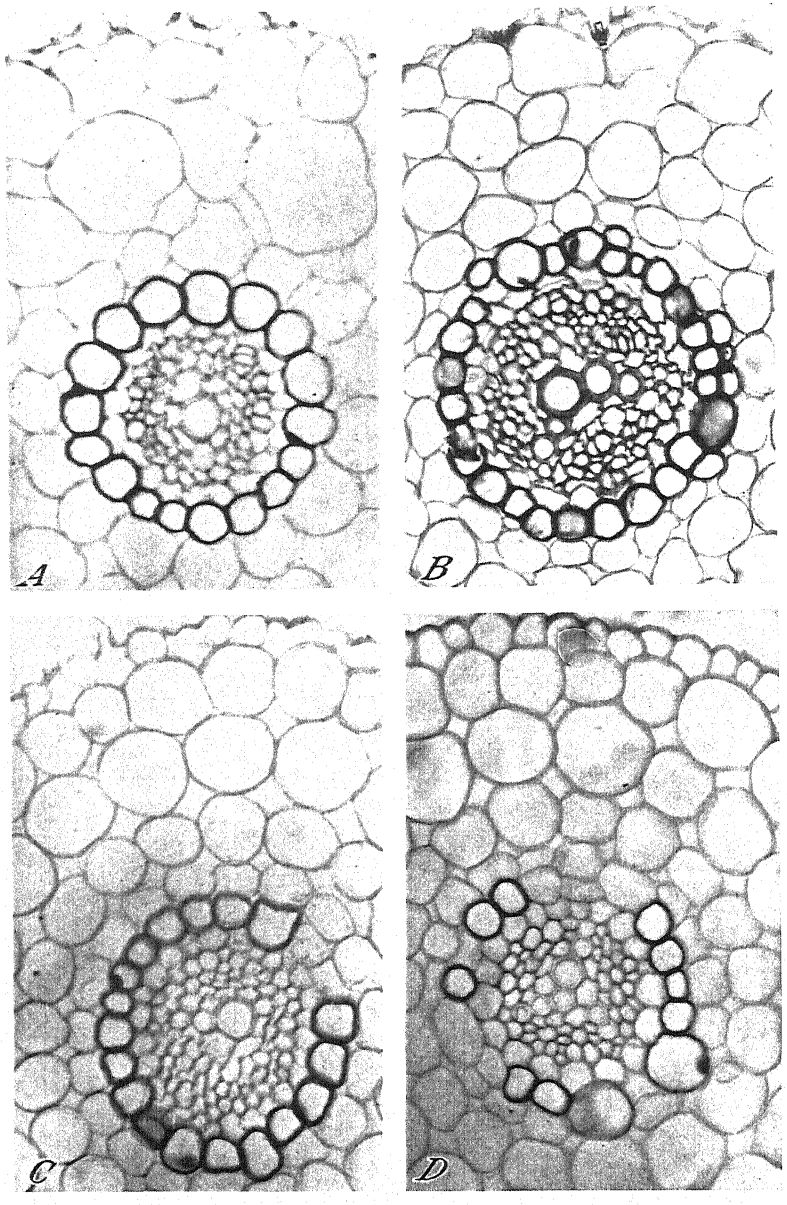
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A, Cross section of lateral rootlet with emerging tertiary root. The thickened endodermal cells form an unbroken semicircle. *B*, Thick-walled endodermal cells of tertiary root continuous with those of parent root which has a broken type of endodermis. Both × 450.



A, Transverse section of lateral rootlet with unbroken type of endodermis. *B*, Unbroken thick-walled endodermis two cells wide in places. *C*, Partially broken type of thick-walled endodermis. *D*, Broken type of thick-walled endodermis. Note that the thin-walled cells lie opposite the protoxylem points. All × 450.

although a complete phellogen may occasionally be formed, a periderm is never developed.

The protoxylem plate of the taproot is diarch. In the lateral rootlets such plates are not uncommon, but the bulk of the laterals is triarch, and in larger rootlets, though not limited to them, tetrarch plates are often found. The protoxylem of the plates of older rootlets

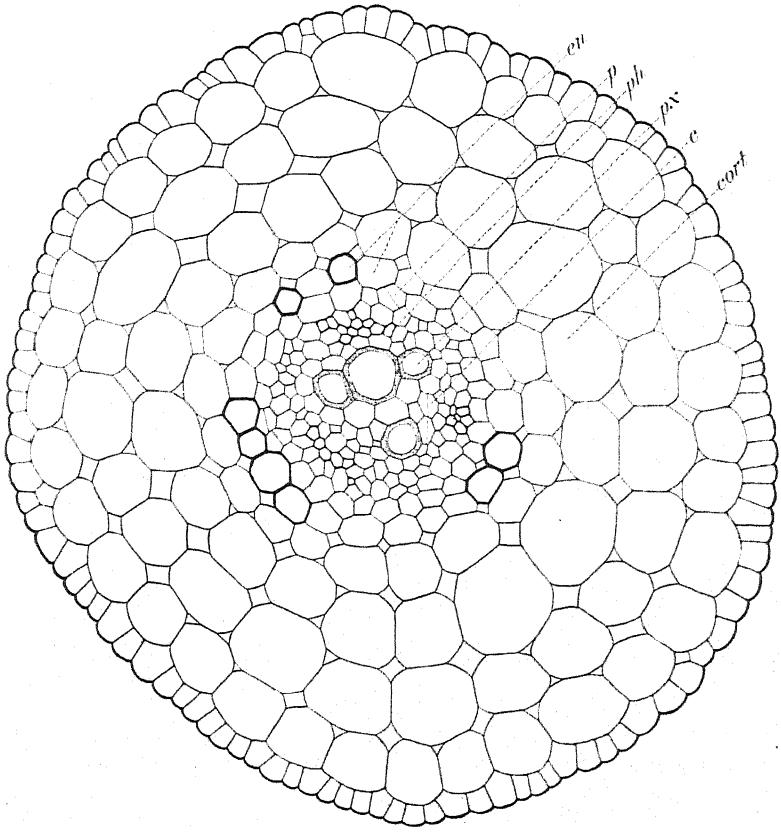


FIGURE 1.—Cross section of lateral rootlet of sugar beet: *en*, Endodermis composed of thick- and thin-walled cells; *p*, pericycle (some of the cells have divided tangentially to form a partial phellogen); *ph*, phloem; *px*, protoxylem; *c*, cambium; *cort*, cortex. × 340.

is augmented by metaxylem elements that sometimes give the central part of the rootlet the appearance of a solid xylem core.

The endodermis is composed of a single layer of axially elongated cells which form a complete jacket around the central stele. The cells have the characteristic Casparian strips and in addition develop secondary thickenings which are of cellulose and stain intensely with Delafield haematoxylin. These secondary thickenings may be present in all endodermal cells (pl. 2, *A*), or they may be absent from certain sectors (pl. 2, *C*, *D*); sometimes they are altogether wanting. Differences in the patterns formed by the thickened cells result in three types of endodermis, as follows: (1) The "unbroken" type, designated in the tables as α, where the thickened cells form axially

uninterrupted rows; (2) the "slightly broken" type, designated as β, where there is an occasional interpolation of an unthickened member; and (3) the "broken" type, designated as γ, where there is a substitution of additional thin-walled cells. In the latter type the thin-walled cells usually lie opposite the protoxylem points (fig. 1 and pl. 2, *D*), but this relation does not always hold true. In the broken type of endodermis there is often a great difference in the sizes of the endodermal cells (pl. 2, *D*), a difference that is the more striking the greater the number of thin-walled cells. The ring of thickened endodermal cells is sometimes augmented by the development of secondary thickenings in adjacent cortical cells (pl. 2, *B*). At times secondary thickenings may even be found in certain pericyclic cells. Where branch roots depart from a lateral, a continuity of the thickened endodermal cells of the parent and branch root is usually maintained (pl. 1, *B*).

EFFECT OF ENVIRONMENT ON LATERAL ROOT SYSTEM

The influence of the preceding crop in the rotation on the lateral root system of the sugar beet was studied on material from plots that had previously been planted to corn, Sudan grass, *Sesbania*, or cowpea, or that had been left fallow. For data on the influence of unfavorable environment, plants were selected from waterlogged spots, poor sandy corners, and from areas in the plots in which the plants showed heavy infestation with plant lice on the young leaves. Most of the plants where growth conditions were unfavorable showed a yellowish discolored foliage and poor top growth. Samples were taken in March after resumption of active vegetative growth and again just before bolting.

Macroscopically, the root systems of the beets from the different plots were much alike, save for individual variation. When top growth was compared, one was struck by the fact that the plants from the rather poor-looking plots, such as those previously cropped to corn, had a root system equal and at times superior to those from the vigorous cowpea or fallow plots. Since the beets from the corn plot at time of bolting equaled those from the other field plots in vigor, it became quite evident that the well-developed root system of the beets from the corn plot was in a large measure responsible for their rapid subsequent development, notwithstanding their poor top growth showing during fall and early spring.

However, the factors that influenced grass top and root development had no appreciable effect on the inner root structure, as may be seen from a study of table 1. Taking as a criterion the large healthy plant with big taproot, no. 4 of table 1, the diameter of the laterals averaged a little more than 300μ, while the ratio of diameter of rootlet to diameter of the stele inclusive of the endodermis was approximately 2.51:1. The rootlets from the plot in which corn preceded sugar beets had a slightly larger stele, and the ratio of diameter of rootlet to diameter of stele was 2.70:1. This was a somewhat lower ratio than that observed in the rootlets from the cowpea or fallow plots.

The rootlets from plants growing under unfavorable environmental conditions gave less uniform data. However, the differences in structure were only quantitative in character, since pathological

disturbances, so often associated with abnormal environment, had no influence on the general make-up of the roots.

The majority of the rootlets, from whatever source, had three protoxylem points. Roots with predominantly diarch plates were observed on only one occasion (table 1, item 5). They came from a rather unfavorable location and often exhibited pathological disturbances in the phloem and adjacent tissues. The ratio of diameter of total root to diameter of stele, however, was approximately the same as that of roots from a normal environment.

TABLE 1.—*Measurements and microscopic data on the lateral roots of sugar-beet plants: Summary of data*

No.	Material: Description	Samples	Average diameter: Entire root		Average diameter: Stele		Ratio of total diameter of rootlet to that of stele	Predominant protoxylem points	Frequency of endodermis pattern			Remarks
		Number	μ	*S.E.*	μ	*S.E.*		*Number*	α	β	γ	
1	With corn as preceding crop.	15	333	±8.6	123	±4.6	2.70:1	3	6	6	3	
2	With cowpea as preceding crop.	13	343	±8.4	119	±5.0	2.88:1	3	5	6	2	
3	After fallow	10	355	±11.1	115	±5.1	3.08:1	3	5	2	3	
4	Large healthy plant with big tap root.	16	304	±12.8	121	±7.4	2.51:1	3	1	3	12	
5	Small yellow plant from sandy corner of field.	11	325	±19.3	128	±5.0	2.54:1	2	9	2	0	Many roots necrotic.
6	Plant with yellow leaves and infested with plant lice.	25	294	±11.5	123	±5.9	2.39:1	3	4	11	10	
7	Small plant from poor stand in low spot.	12	225	±13.0	83	±4.1	2.71:1	3	3	4	5	
8	Medium-sized yellow plant from waterlogged spot.	24	281	±31.2	90	±3.8	3.12:1	3	1	12	11	About 25 percent of the roots necrotic.

The three types of endodermis, as illustrated in plate 2, *A*, *C*, and *D*, and referred to in table 1 as endodermis patterns α, β, γ, were found in all root material, regardless of its source. Random observations on cross sections showed a prevalence of one or the other type, but this was seldom sufficiently definite to be significant. Roots from an apparently normal environment (from corn, cowpea, and fallow plots) showed the closed and broken types in fairly equal proportions, while the roots from plants growing in an unfavorable environment showed a preponderance of the broken type (table 1). An exception again is found in the roots from a plot previously noted as having mostly diarch protoxylem plates (table 1, item 5), in which the vascular cylinder was almost always jacketed with an endodermis of unbroken thick-walled cells.

PATHOLOGICAL DISTURBANCES

It would be strange, indeed, if among the large number of rootlets belonging to a plant only normal individuals should be found; and it is noteworthy that in the case of the lateral rootlets of sugar beets growing in what should be considered a normal environment the percentage of diseased individuals is so small. If diseased rootlets are present at all, the type of injury is usually slight and often mechanical in nature, being limited to a destruction of small sectors of cortex and

rarely reaching the vascular tissue. At times, the entire cortex is more or less collapsed while the vascular tissue is still normal and capable of conducting water and nutrients. True pathological disturbances in the vascular tissue are sometimes found in the protoxylem cells; these are seen filled with a gummy deposit, or may even contain micro-organisms.

In plants grown under seemingly disadvantageous conditions, especially when temporarily subjected to high water during the winter months, the number of diseased rootlets may be quite large and the pathological picture fundamental and severe. Diseased xylem cells become quite common and are often accompanied by pathological changes in the water-conducting cells or even by a diseased condition of the phloem (pl. 3, *A*). Phloem necrosis in its inception is limited to a few pericyclic cells, those elements that were shown to be so susceptible to virus invasion.[5]

The diseased pericyclic cells are usually few and do not hypertrophy like those affected by the curly top virus. At times they separate and a red gummy deposit is found lodged in the intercellular space formed between the cells of the endodermis and those of the pericycle. Usually, however, necrosis spreads from the pericycle to the phloem and the adjoining parenchyma cells, involving all phloem groups or being limited to one or two, depending on whether the root in question is diarch or has a larger number of protoxylem points. The necrotic lesions somewhat resemble those associated with the curly top disease, but, fundamentally, they rather belong to the type found in potato plants affected with the leaf roll disease.[6]

Necrosis in some of the rootlets has a stimulating effect on the phellogen of the still healthy sectors, causing the production of a partial multilayered periderm (pl. 3, *B*). A similar effect may also be noted on the normal cambium below the diseased phloem.

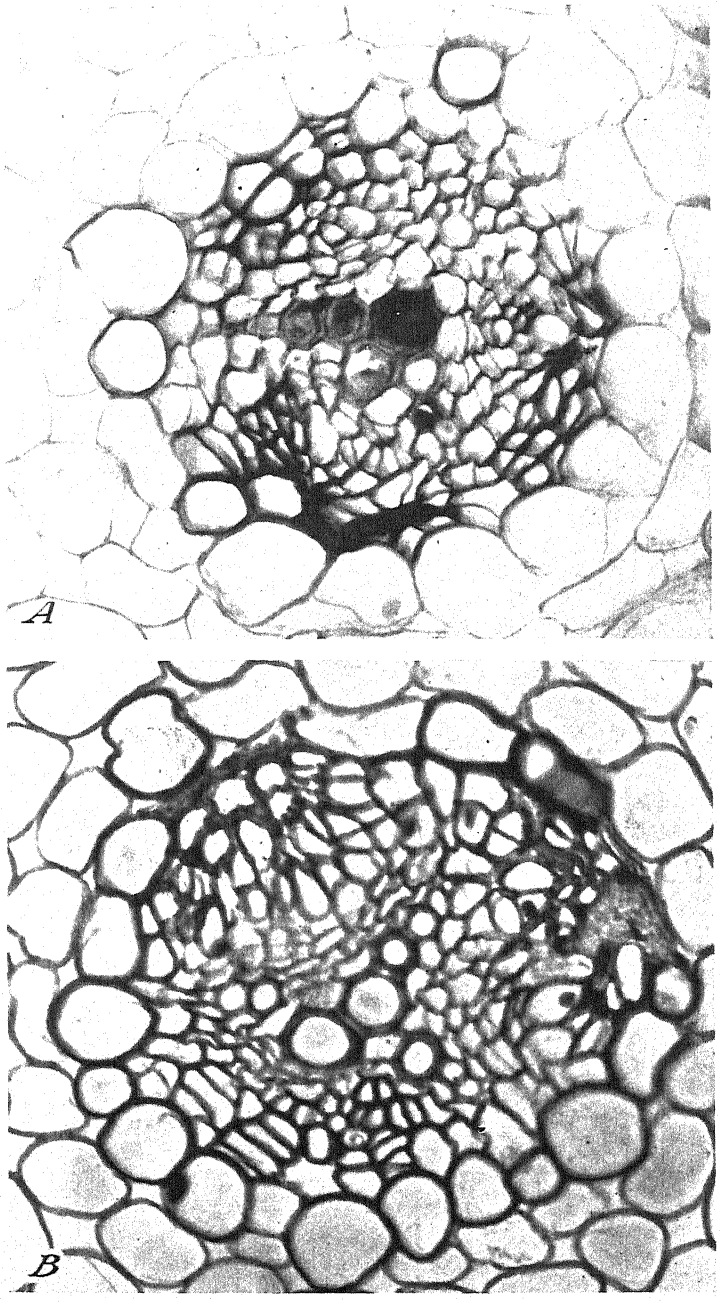
SUMMARY

Under normal conditions, the lateral rootlets of the sugar beet develop no secondary tissues and the protoxylem plates are predominantly triarch. The cells of the endodermis often develop secondary thickenings, forming an entire or partly broken thick-walled jacket around the vascular cylinder. If the thin-walled cells are few in number, they may well be likened to transfusion cells which are so common in a thick-walled endodermis.

The preceding crop in the rotation, while inhibiting early top growth in some plots (noticeably in beets after corn), apparently did not affect root development; e. g., the sugar beets from the "after-corn" plot, characterized by less top growth, showed a root system equal to that of the luxurious "after-cowpea" plot. No significant structural differences were noted in any of the material coming from an apparently normal environment; but distinct pathological changes, especially in the nature of phloem necrosis, were observed in plants grown under disadvantageous soil conditions.

[5] ARTSCHWAGER, E., and STARRETT, R. C. HISTOLOGICAL AND CYTOLOGICAL CHANGES IN BEETS AFFECTED WITH CURLY TOP. Jour. Agr. Research 53: 637-657, illus. 1936.

[6] ARTSCHWAGER, E. OCCURRENCE AND SIGNIFICANCE OF PHLOEM NECROSIS IN THE IRISH POTATO. Jour. Agr. Research 24: 237-245, illus. 1923.



A, Diseased lateral rootlet showing deposit in protoxylem cell and phloem necrosis. There is much variation in the size of the endodermal cells, most of which are thin-walled. *B*, Transverse section of diseased lateral rootlet. The rootlet is triarch with two of the phloem groups necrotic; above the third phloem group is a well-developed several-layered periderm. Both × 750.

EFFECT OF THE DWARF DISEASE ON THE ALFALFA PLANT[1]

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INTRODUCTION

In previous papers (*5*, *6*, *7*)[2] the writer has discussed the nature of the dwarf disease of alfalfa (*Medicago sativa* L.) and the effect of certain environmental factors on its spread and development. That the disease causes a considerable disturbance in the normal histology and physiology of the alfalfa plant is indicated by the dwarfing of the tops and the extensive discoloration in the roots. The results of certain histological and physiological studies made to obtain a better understanding of the nature of the effect of dwarf on the alfalfa plant form the basis of this paper.

HISTOLOGICAL METHODS

For the most part fresh material was used, although many roots and stems were fixed in formal-acetic alcohol, embedded in paraffin, sectioned, and stained. Some material was stored in fixative and sectioned with the sliding microtome without being embedded in paraffin.

The varieties studied were Hairy Peruvian and Chilean, and the plants were collected in the field at different times of the year.

Various stains were used, but Heidenhain's iron-alum haematoxylin and carbolfuchsin were the most helpful. Certain points, such as the yellowing of the vessels, could be studied best without staining.

THE DISEASE IN THE ROOT

When the bark is removed from diseased taproots in the winter and spring the yellow color observed during the growing season (*5*) usually is found to be more or less overgrown with a thin layer of new white wood (fig. 1). With the advent of cool weather in the autumn the disease becomes less active and gum[3] formation ceases, so that by late spring a layer of white wood 1 mm or more in thickness is present between the cambium and the yellow wood produced during the previous summer. The thickness of this new layer varies greatly in different roots and in different parts of the same root. A relatively thick layer of new wood may form on one side of the root, where the disease has not made such inroads, and little or none on the other side. A macroscopically perceptible layer of new wood may be present as early as December. The disease becomes active again the next

[1] Received for publication Oct. 3, 1936; issued August 1937. Cooperative investigations of the division of Forage Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture, and the division of Agronomy, California Agricultural Experiment Station.

[2] Reference is made by number (italic) to Literature Cited, p. 104.

[3] The term "gum" is used to designate the material plugging the vessels, regardless of its chemical composition.

spring and by midsummer has involved much of the new wood, and the plant slowly succumbs.

A microscopical study of cross sections of diseased roots shows clearly the parts affected. Little or no evidence of trouble of any kind appears in the bark, cambium, wood rays, or fibers of affected plants until the late stages of the disease, when there is usually some yellowing caused by a soluble stain. The parenchymatous cells contiguous to or near plugged vessels are sometimes affected, as is shown by the yellow color in their walls and their staining reaction. Sometimes there is a small amount of plasmolysis in these cells also. How-

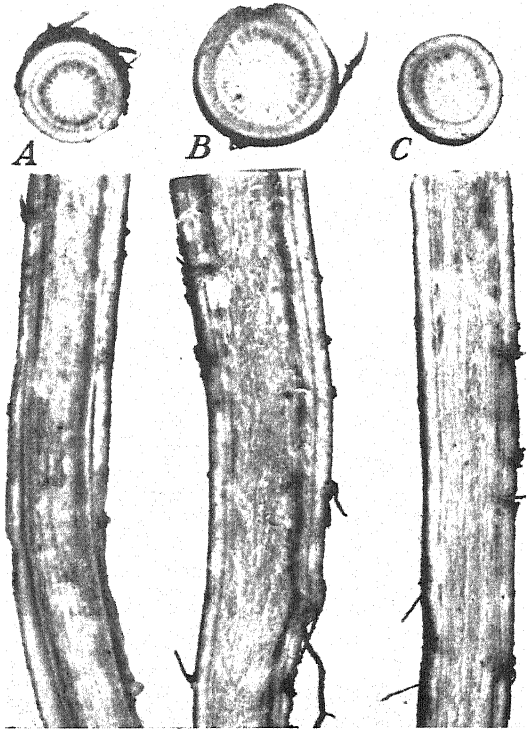


FIGURE 1.—Cross and longitudinal sections of three alfalfa roots affected with dwarf. The dark-colored streaks are regions where the vessels are plugged with gum. These roots show the early spring condition, when the discolored areas are covered by a layer of new growth. In cross section *A* the affected tissues form a complete dark-colored ring about 1 mm beneath the cambium. Cross section *B* shows a much wider band of diseased cells on the right and upper sides than elsewhere, and cross section *C* shows only a very small discolored region in the upper left-hand part. Compare with figure 3, which shows not only the unaffected region of winter growth covering the affected ducts beneath but also the layer of plugged cells nearer the cambium, developed during the current season. About natural size.

ever, in many cases, at least in the earlier stages of the disease, the parenchyma cells stain and appear quite normal. No X-bodies have ever been seen; the visible evidence of the disease is restricted for the most part to the vessels of the xylem.

Not all of the xylem is affected. The plugging at first may be limited to one or a few ducts in one or more bundles. Even in fairly advanced stages of disease certain bundles may be entirely unaffected while others are badly plugged (fig. 2). In fact, usually only a com-

paratively small proportion of the total number of ducts of a bundle are involved (figs. 2 and 3). The affected region in roots in which the disease is most active, as indicated by the formation of gum, lies just within the cambium; that is, in the part of the xylem that is functioning most actively in transporting water and nutrients. In advanced stages of the disease so many of the ducts in this region may be plugged that water cannot reach the tops in sufficient quantity to prevent wilt-

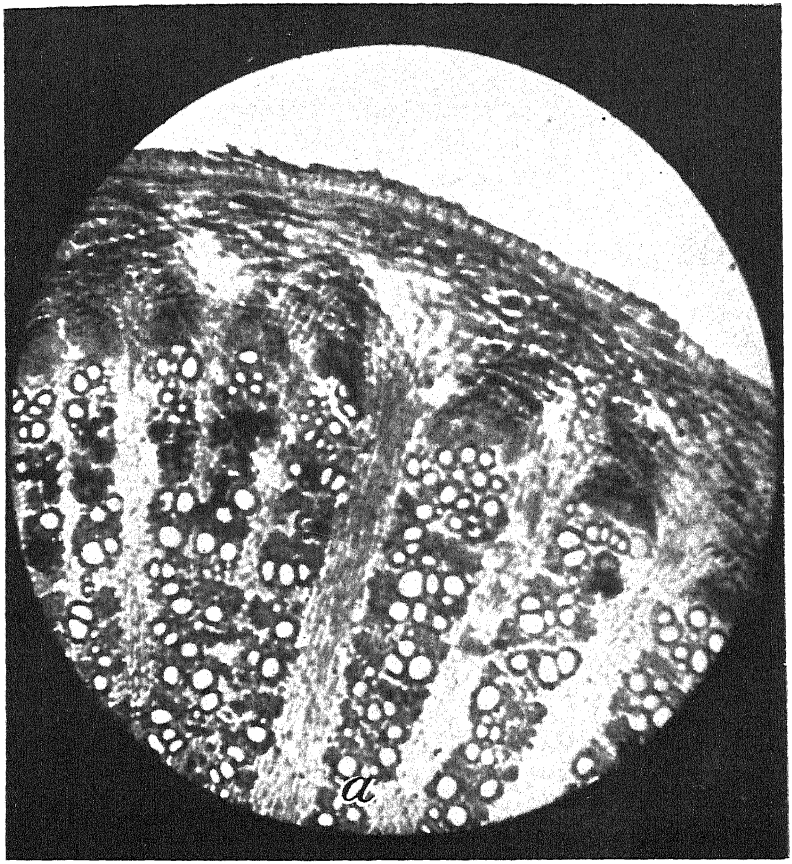


FIGURE 2.—Cross section of alfalfa root having medium stage of dwarf. One bundle (*a*) has no plugged vessels, while the adjacent bundle on the left has several and the one on the right has two. Although unaffected bundles are often found, more frequently some ducts in all the bundles are more or less plugged, especially in the later stages of the disease. The affected region is several cells back from the cambium, a condition commonly seen during the winter and spring months. × 35.

ing during the middle of the day in bright weather even when the soil moisture is very high.

The youngest vessels next to the cambium may be plugged but usually they are not except in the late stages of the disease. The plugging is most commonly in the second and third layer of ducts from the cambium. The width of the affected region increases as the disease develops. In the later stages the affected area usually occupies a zone not more than 2 or 3 mm in width, or, roughly, the outer third

of the distance from the center of the root to the cambium (fig. 3). Not much consistency is shown in the way the ducts are plugged. Sometimes the larger, sometimes the smaller, and sometimes all the ducts are involved. A single plugged duct or a group may be surrounded by ducts that apparently are unaffected. Likewise, one bundle may have a number of plugged ducts while an adjacent one

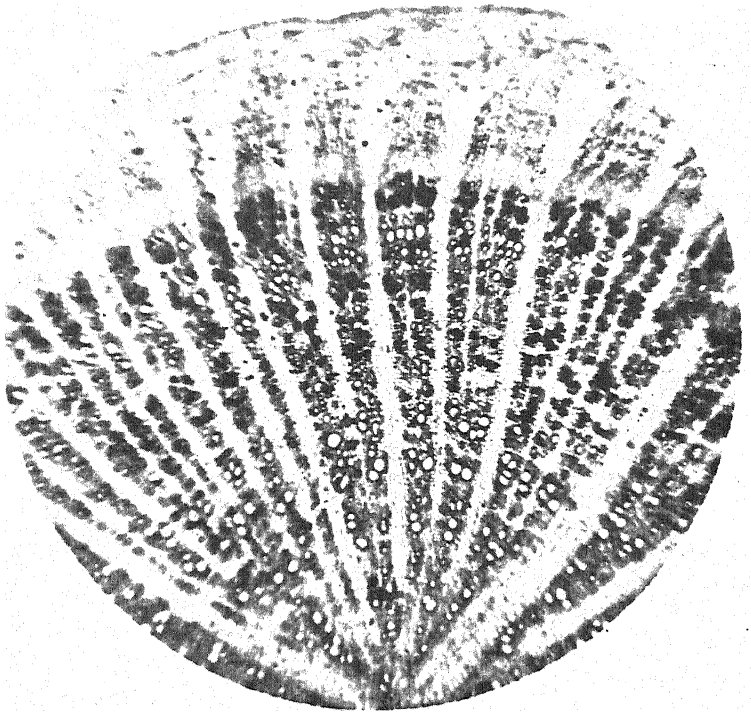


FIGURE 3.—Cross section of alfalfa root having late stage of dwarf. Three distinct bands can be distinguished in the outer half of the wood, two in which the vessels are plugged with gum, separated by a third which is almost unaffected. The band next to the cambium, which is the current season's growth, contains a large percentage of plugged ducts. Beneath this layer is the winter growth, in which the ducts are largely free of gum, although all or nearly all of the ducts in some bundles appear to be plugged. The previous season's growth, just beneath, also is badly affected. × 35.

has only a few or none at all (fig. 2). Many ducts lying end to end may be plugged, forming long strands of apparently continuous gum. The gum often shrinks and pulls away from the walls. Tyloses are sometimes present in the ducts.

THE DISEASE IN THE STEMS

It was pointed out in a previous paper (*5*) that the tops of affected plants show a gradual dwarfing which continues until the plant dies. It might be expected that the yellowing and plugging of the vascular system of the diseased roots would extend into the stems, but a study of many sections of stems has shown little if any stain and only a

small amount of plugging. Such little plugging as there is in the vessels of the green stems is found chiefly in the late stages and then only for a distance of an inch or two above the crown (fig. 4). The vessels of the stem that are plugged are usually though not always near the pith. The lack of extensive plugging in the stem tissue may be due, at least in part, to the fact that the disease progresses very

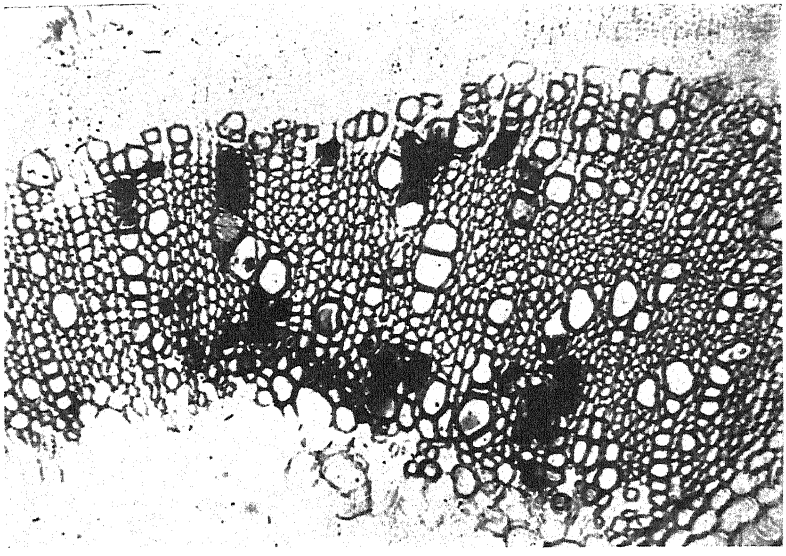


FIGURE 4.—Cross section of a portion of an alfalfa stem affected with dwarf. Several plugged ducts are evident, but plugging of the vessels in the stems is not extensive. Only the xylem is clearly shown. The pith is at the lower part of the picture. × 150.

slowly and that the stems are cut frequently (once every 28 to 34 days) during the growing season, when the disease is most active.

CHARACTER OF THE GUM

The gum may be homogeneous in character (figs. 5 and 6); it may have what appears to be a flocculent or granular precipitate; or it may contain numerous coccuslike or rod-shaped bacterialike bodies (fig. 7). The homogeneous and granular gum is comparable to that formed in many other plants. The bacterialike bodies are not present in the ducts of all affected plants; in fact, they have not been found at all in about 25 percent of the plants examined. Nevertheless they constitute one of the most reliable microscopical diagnostic characters of the disease.

From the beginning of these investigations the question whether or not these bodies are bacteria has presented itself. All efforts to prove by cultural methods that they are living entities have failed (7). Some bacteriologists and pathologists who have examined the material have unhesitatingly pronounced these bodies bacteria; others equally competent to judge have said that they were not bacteria. These bodies are limited to the ducts of the xylem. Pathogenic bacteria usually, if not always, sooner or later pass out of the vessels into the intercellular spaces of the parenchyma. The bodies in question are

embedded in a gum which holds them in clumps and keeps them from undergoing Brownian movement. When this gum is dissolved away by 50-percent chromic acid the bodies float free, showing that they are individuals and have a solubility slightly different from that of the gum. At times they appear yellow like the gum in which they are embedded.

The outer portion of these bacterialike bodies differs from the interior as seen both in stained and unstained preparations. The outer portion takes the stain while the inner remains largely or entirely unstained. This has been interpreted by some authorities as indicat-

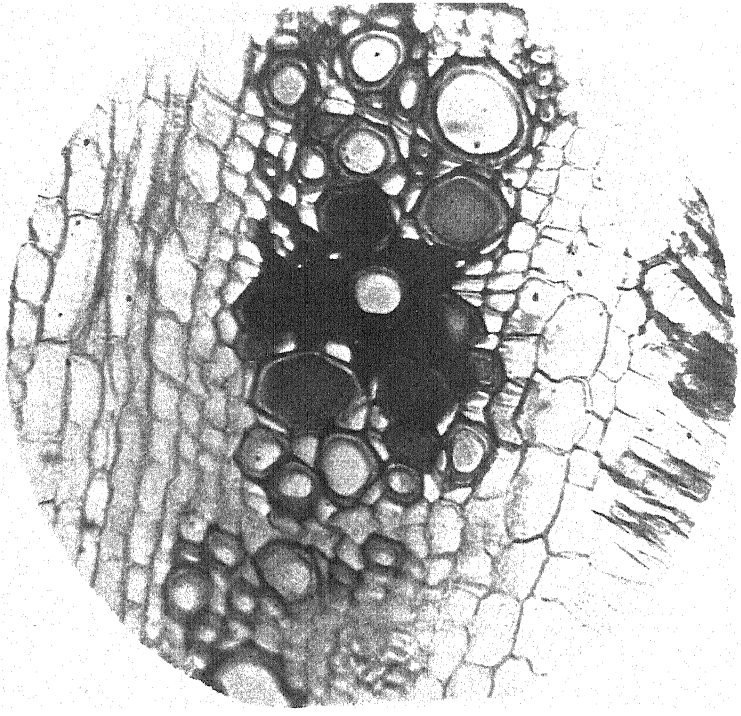


FIGURE 5.—Cross section of alfalfa root affected with dwarf showing group of plugged vessels in which some of the walls have been so changed or impregnated with gum that they have practically lost their identity. Adjacent wood parenchyma cells have been affected to a limited extent. × 190.

ing the presence of a spore (figs. 7 and 8). In certain stages this outer portion takes a red color when treated with phloroglucin and hydrochloric acid, just as does the gum, indicating its similarity to wound gum. Neither these bodies nor the gum takes on this color in the early hyaline stage. So far as ascertained, no bacteria give this reaction for wound gum. Of course, it is possible that the outer portion of these bodies is impregnated with gum and so gives the characteristic gum reaction to the phloroglucin-hydrochloric acid treatment. These bodies are gram-negative and do not take many of the stains that most bacteria do, but rather stain like the gum

near or surrounding them. This fact suggests that these bodies are of a material similar to gum but that they differ from it slightly, at least in their solubility in chromic acid.

In the early stage these bodies are embedded in a hyaline material more or less creamy or semifluid in consistency. This was determined by stirring the material in a single duct under the microscope by means of a capillary glass needle manipulated by an apparatus similar to that described by Roberts (*4*). This material very soon hardens so that it can be moved about in a cell as a unit and the needle cannot penetrate it. Later the contents of the vessel turn yellow and give the wound-gum reaction as previously described. In the semifluid

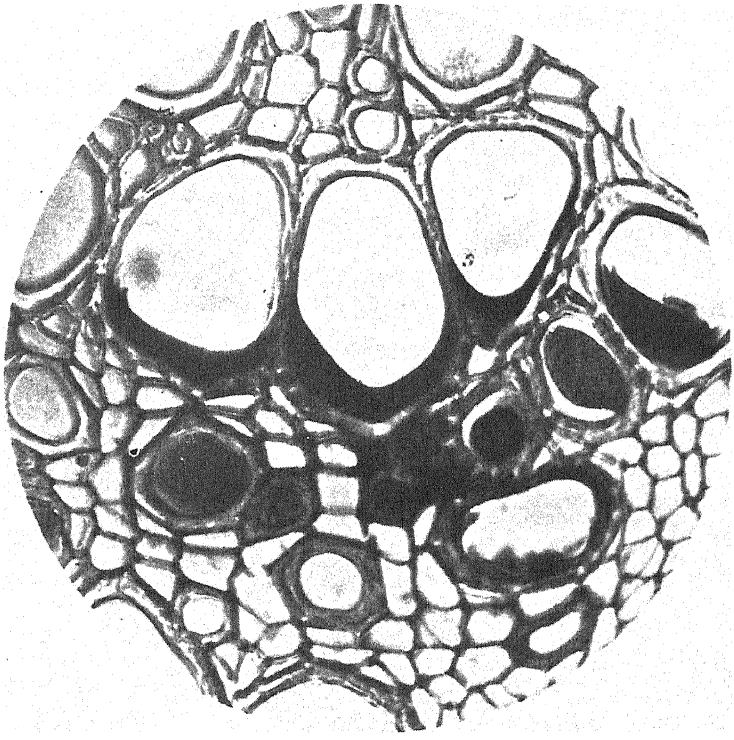


FIGURE 6.—Cross section of alfalfa root affected with dwarf, showing some ducts completely filled and others only partly filled with gum. For the most part the surrounding cells show no signs of disease, although there is a fairly profuse gumming just below the center one of the three large vessels. The upper parts of the walls of these three cells appear to be unaffected even though considerable gum has been formed lower down. × 370.

state the contents of the ducts are more or less readily washed away in the staining process. A few of the bodies were sometimes found isolated, lying along the wall of the duct and apparently free of gum. These often gave the red color when treated with phloroglucin and hydrochloric acid, which suggests that this reaction is due to the nature of the bodies themselves and not to the gum in which they are embedded. These bodies give a negative reaction for tannins, chitin,



FIGURE 7.—Cross section of alfalfa root stained with carbolfuchsin, showing bacterialike bodies. These may be so numerous that they entirely plug the lumen of the vessel. × 1,100.



FIGURE 8.—Longitudinal section of a vessel in an alfalfa root stained with carbolfuchsin, showing bacteria like bodies scattered about. × 1,200.

protein, and fats and do not react to polarized light as crystals do. Knowledge of their exact chemical nature will have to await further study.

ORIGIN OF THE GUM

To determine with certainty the source of the gum plugging the vessels of alfalfa plants affected with dwarf is difficult. In the early stages of the disease certain portions of the walls become impregnated with gum, which eventually collects along their inner margins (figs. 9 and 10). Later the ducts may be partly or completely filled with

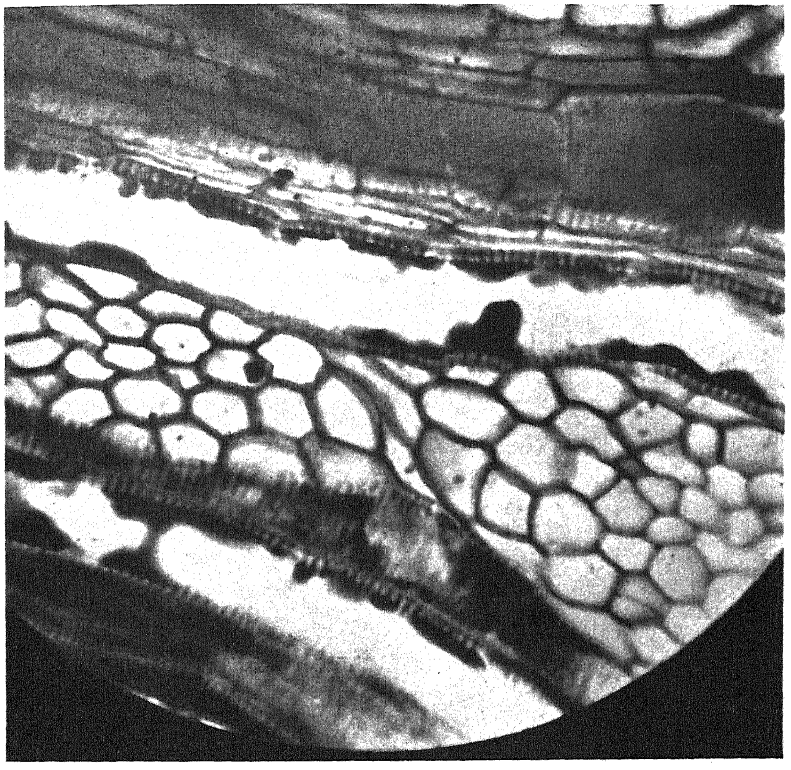


FIGURE 9.—Tangential longitudinal section of alfalfa root showing two vessels each with small amounts of gum projecting into the lumen from the walls. In some cases the gum has taken the form of globules and in others it has formed a thin layer covering several pits. Note that the gum is absent from or at least is not apparent in the parenchyma beneath the upper vessel. × 260.

gum (figs. 2, 3, 5, and 6). The most probable explanation of the origin of this gum is that it is formed in the adjacent parenchyma cells and is forced through the pits into the ducts. There is sometimes evidence of a slight change in the affected vessel wall or in the region of the middle lamellae, but this is so restricted that it hardly seems probable that it serves as a source of much if any of the gum. The walls are never completely disintegrated and gum pockets are never formed as happens in many diseased plants. It seems more probable that sugars, starches, or other carbohydrates in solution in the cell sap in the adjacent parenchyma cells are the source of the gum.

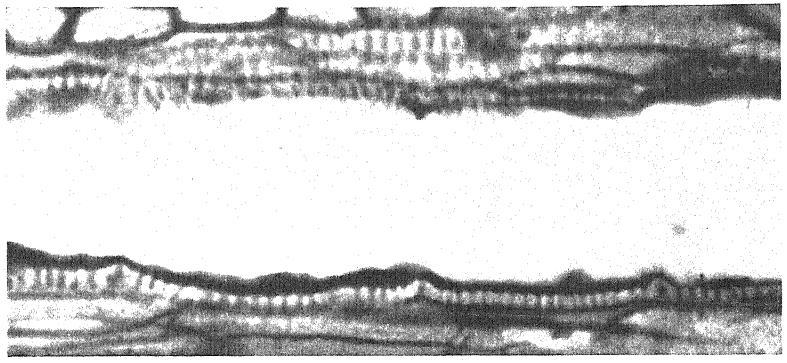


FIGURE 10.—Another vessel, from the same slide as the vessels in figure 9, showing a long sheet of gum along the wall. The gum in the early stage is semifluid so that in most cases it spreads out in a thin layer along the wall. × 300.

WATER CONTENT OF DISEASED PLANTS

The comparative amount of water in diseased and healthy alfalfa plants was determined in an experiment set up on August 20, 1931. From a field of Chilean alfalfa in its fourth year, diseased and healthy plants having about half of their blossoms open were selected. The plants all came from the same place in the field, and therefore should have been comparable in every way except for the presence of the disease in some of them. Five plants in a fairly late stage of the disease and five healthy plants were dug, wrapped well in newspaper to prevent the loss of water, and brought to the laboratory. After the adhering soil had been removed from the roots with a brush and cloth without wetting, composite samples were made of the healthy tops, diseased tops, healthy roots, and diseased roots. The roots and tops were cut into small pieces, placed in tared weighing bottles, weighed, dried to constant weight at 100° C., and reweighed. The field had been irrigated 2 days before the plants were dug, and the soil was still quite moist. A second experiment, conducted on August 25, 1931, was an exact duplicate of the first except that the tops had been cut in the meantime and the stems were only 3 to 4 inches tall and consequently were young and succulent. The soil was damp, as the field had been irrigated the preceding day. Only the new stems and leaves and about a foot of the taproots were used. This was true for both experiments.

On September 1, 1931, six diseased plants and six healthy plants from the same field were treated in the same manner as in the two preceding experiments except that the plants were held with their taproots in water for 24 hours before being prepared for the dry-weight determinations. The tops were turgid at all times. In this experiment the yellow crown tissue was placed with the roots, and all green tissue was included with the tops. The affected plants were in various stages of disease, but in all cases the disease had advanced sufficiently to cause more or less dwarfing of the tops. The healthy tops were about 1 foot to 15 inches tall. This experiment was duplicated on September 10 and again on September 16, 1931. Of course, the stage of top growth was not the same in any of these tests. On September 1 the tops were perhaps not more than half grown, while

on September 16 they were in what is usually classed as the bud stage, although there was an occasional partly open blossom.

The percentage of water in the roots and tops in these experiments is given in table 1. These data show that on each date the water content of the healthy tops was slightly higher than that of the diseased tops, while the reverse was true for the roots. The greatest difference between the moisture content of the diseased and healthy tops was on August 25, when the top growth was young and succulent. The greatest difference between the water content of the diseased and healthy roots was in the August collections. No explanation for this can be offered, unless it is the fact that the roots of the September samples stood in water before they were prepared for drying. The difference between the average percentage of water in the diseased and healthy tops and roots for all experiments is quite small and probably is not significant. Nevertheless, the tops of the healthy plants were considerably larger than those of the diseased, and it may be that the larger tops withdrew slightly more water from the roots during the period which of necessity elapsed between the removal of the plants from the soil or water and the determination of the wet weight. Perhaps the slightly lower water content of the diseased tops was due to a partial water deficit. That such a deficit does exist at times is suggested by the fact that diseased plants often wilt even when growing in wet soil.

TABLE 1.—*Average percentage of water in healthy and dwarfed alfalfa tops and roots on different dates in 1931*

Date	Average percentage of water in—			
	Tops		Roots	
	Healthy	Diseased	Healthy	Diseased
Aug. 21	70.1	68.5	69.5	74.4
Aug. 25	82.5	77.2	66.2	70.9
Sept. 1	82.2	79.2	70.5	70.7
Sept. 10	82.7	80.6	68.8	69.3
Sept. 16	77.8	75.2	67.4	70.1
Average	80.0	77.3	68.7	70.5

TRANSPIRATION

A study was also made of the rate at which dwarfed and healthy plants transpire. Twelve 4-year-old Chilean alfalfa plants, six dwarfed and six healthy, which had received the same treatment throughout, were used for each experiment. The plants were collected at about the same time in the morning (8 a. m.), wrapped well in newspaper, and taken to the laboratory, where the roots were placed at once in water. As much of the taproot as could be pulled conveniently, usually about 8 to 12 inches, was left on each plant. The length of the tops varied in the different experiments. The healthy tops ranged in height from 15 to 16, 14 to 18, and 26 to 31 inches in the experiments conducted on September 1, 10, and 16, 1931, respectively. The tops were of different ages and different stages of maturity, since they were all cut on August 24. The healthy

plants had reached the bud stage by September 16, when the last experiment was conducted. As soon as possible after the plants were brought to the laboratory the taproots and crowns were washed free of adhering soil, and after the lower end of the taproots had been cut off, taproots and crowns were placed at once in 100-cc graduated cylinders filled with tap water. Sufficient water was added to or withdrawn from the graduates to bring the water level to the 100-cc line. The graduates were placed on a table in two rows, so arranged that the tops of the plants did not touch and no two diseased or healthy plants were adjacent. In this way it was hoped to have the diseased and the healthy plants subjected to the same average conditions. The room temperature ranged from about 22° to 30° C. during the experiment, and there was some air circulation from open windows, but no wind was blowing directly upon the plants. The space between the roots and the mouth of the graduate was left open, since preliminary trials showed that not enough water evaporated from the surface in 7 hours to vitiate the results.

After the graduates had been filled as described, a measured amount of water was added each hour to bring the level back to the 100-cc mark. In this manner the total amount of water transpired by each plant in 7 hours was determined. The averages of these values, each representing the average for six plants, for the three experiments are given in table 2. Considerable variation was shown in the amount of water transpired both by the different plants and from hour to hour by the same plants. As might be expected, the greatest water loss occurred during the warmest part of the day.

TABLE 2.—*Comparative rate of transpiration by dwarfed and healthy alfalfa plants*[1]

Average amount of water transpired per plant in 7 hours		Average volume of tops		Average dry weight of tops		Average amount of water transpired in 7 hours				Ratio of diseased to healthy	
						Per cubic centimeter of green top		Per gram of dry top			
Healthy	Diseased	Healthy	Diseased	Healthy	Diseased	Healthy	Diseased	Healthy	Diseased	Volume basis	Dry-weight basis
Cc	*Cc*	*Cc*	*Cc*	*G*	*G*	*Cc*	*Cc*	*Cc*	*Cc*		
67.1	28.8	85.3	68.6	10.7	7.5	0.80	0.42	6.27	3.84	0.53	0.61
58.1	23.3	74.5	50.0	8.3	5.8	.78	.47	7.00	4.02	.60	.57
66.4	26.7	99.5	53.7	15.5	8.4	.67	.50	4.28	3.18	.75	.74

[1] 6 dwarfed and 6 healthy plants were used in each of the 3 experiments.

The data presented in table 2 show that the healthy plants transpired more rapidly than the diseased plants in all experiments. The tops, however, varied considerably in size; hence, to get the comparison on a more uniform basis, the volume of tops as well as their dry weight was determined. The volume of the tops was first obtained by the water-displacement method, after which they were dried to constant weight in an oven at 100° C. When the volume of tops is used as the criterion, a comparison of the rate of transpiration in the three experiments shows that the healthy tops transpired 0.80, 0.78, and 0.67 cc, and the diseased tops 0.42, 0.47, and 0.50 cc per cubic centimeter of tops, respectively. The corresponding values, obtained

by using the grams of dry weight as a basis of comparison, are 6.27, 7.00, and 4.28 cc for the healthy plants and 3.84, 4.02, and 3.18 cc, for the diseased plants, respectively. When the ratio of diseased to healthy plants was calculated, the values in the last column were obtained. These values show a fairly close agreement between the results obtained by the two methods. In general, they indicate that the diseased plants transpired on an average only 53 to 75 percent as fast as the healthy plants, or, expressed in another way, the healthy plants on a volume basis transpired on an average, about 1.6 times as fast per unit of top tissue, as did the diseased plants.

RATE OF WATER FLOW THROUGH DISEASED ROOTS

After it had been determined that alfalfa plants affected with dwarf transpire much more slowly than do healthy plants, the rate at which water could pass through diseased roots was studied. It is known that the tops of diseased plants sometimes wilt during the warm part of the day even when the soil is wet. This indicates that the tops cannot obtain water rapidly enough to offset that lost by transpiration. Likewise, it has been shown by microscopical examination that many of the vessels of affected plants are plugged with gum. That the slowing down of the rate of transpiration and the plugging of the vessels were somewhat interrelated seemed probable.

The rate at which the water can pass through 4-inch segments of the taproots of diseased and healthy plants was determined by a method similar to that described by Melhus, Muncie, and Ho (*3*). Water was pulled through segments of the roots of 4-year-old alfalfa plants under a constant suction pressure. The diseased and healthy plants were obtained from the same place in the field, were collected at the same time, and were held under comparable conditions throughout.

TABLE 3.—*Average amount of water pulled through 4-inch segments* [1] *of healthy and diseased alfalfa roots of different diameters in 10 minutes*

Diameter of root at small end	Healthy roots		Diseased roots		Diameter of root at small end	Healthy roots		Diseased roots	
	Plants	Average amount of water pulled through in 10 minutes	Plants	Average amount of water pulled through in 10 minutes		Plants	Average amount of water pulled through in 10 minutes	Plants	Average amount of water pulled through in 10 minutes
Mm	*Number*	*Cc.*	*Number*	*Cc.*	*Mm*	*Number*	*Cc.*	*Number*	*Cc.*
6	2	19.5	1	4.7	10	3	53.1	2	36.0
7	3	10.1	1	8.4	11	5	61.3	4	23.4
8	5	24.8	4	11.3	12	2	55.7	6	26.4
9	3	24.0	5	24.5	13	2	86.1	2	59.9

[1] Each segment was taken from a different plant.

The plants required for the day's testing were brought to the laboratory in the morning and kept in water until used. For the most part, a diseased and then a healthy plant was studied in order to minimize any error that might be due to a change in the plants on standing in water. The segment of root used was taken just below the crown. The diameter of the smaller end was used as a basis of

comparison, since it was assumed that the maximum amount of water that could pass through the root was limited to the amount that could enter the small end. The number of cubic centimeters of water that passed through the 25 segments of the diseased and the healthy roots in 10 minutes was determined, and the values are recorded in table 3. The data in this table show considerable variation in the amount of water that passed through roots of different diameters. Individual healthy roots of the same diameter seemed to vary considerably in this respect. Perhaps this is to be expected of roots of this age and character. Seldom do any two plants have crowns or tops of the same size, or indeed taproots of the same length. Taproots likewise vary greatly in diameter even in a distance as short as 4 inches.

This experiment, however, had only one object, namely, to compare diseased and healthy plants, and it was thought that if any appreciable difference existed it would be brought out by a study of 25 plants of each group. This proved to be the case, as table 3 shows. Although the differences were sometimes slight, as in the case of the roots 7 mm in diameter, for the most part they were rather striking. In the case of roots 9 mm in diameter, the healthy roots conducted more water than the diseased except for one diseased root, which conducted water more freely than any of the healthy roots and was responsible for the slightly higher average for the diseased roots. That the passage of water through the diseased plants should vary greatly in rate is to be expected, not only in view of the variation found in its passage through the healthy plants but also because of the difference in the stage of disease and consequently in the number of ducts plugged with gum. It was impracticable to use roots entirely comparable in every way, owing to the difficulty of obtaining them and to the impossibility of telling by macroscopic examination the exact amount of plugging in the diseased root. The average amount of water pulled through the healthy and diseased roots was 40.6 cc and 25 cc, respectively. This means that on an average water passed through the healthy root segments 1.6 times as fast as through the diseased roots. The fact that the healthy plants transpired approximately 1.6 times as fast as did the diseased plants and permitted the passage of water 1.6 times as fast as the latter suggests that there is a close correlation between these two factors. The data presented in this paper seem to indicate that the inability of the affected alfalfa roots to conduct water may have much to do with the dwarfing of the tops and the ultimate death of the plant.

HYDROGEN-ION CONCENTRATION AND TOTAL ACIDITY OF HEALTHY AND DISEASED PLANTS

It is a well-known fact that plants contain various acids which are more or less highly ionized. The formation of these acids appears to be closely associated with the vital activities of the plant. Anything that alters appreciably the normal processes of a plant may effect a corresponding change in the acidity of its sap. Hurd (*2*) found that the expressed juice of corn plants varied greatly in both hydrogen-ion concentration and titratable acidity, these being influenced largely by environmental factors which affected the vegetative vigor of the plants. A number of investigators have shown that the hydrogen-ion

concentration of the sap of plants can be changed by applying certain fertilizers to the soil in which they are growing.

Since the rate of growth and transpiration of the alfalfa plant are affected by the dwarf disease, it seemed quite possible that its acidity also would be affected. To test the validity of this assumption, the hydrogen-ion concentration and total acidity of healthy and diseased alfalfa plants were determined. Four-year-old alfalfa plants of the Chilean variety, which had been given comparable treatment throughout, were used. The diseased and healthy plants came from the same area in the field; those for each experiment were collected at the same time and were given the same treatment.

The tops of diseased plants, although of the same age, were not so far advanced as those of the healthy plants. In the first two experiments the tops of the latter had made a vigorous growth and were in the early bud stage, while those of the former were somewhat less advanced, the stage of advancement depending upon the stage of the disease. The method of determining the total acidity was largely that described by Haas (*1*). Twenty-five cubic centimeters of juice from diseased and 25 cc from healthy plants were obtained by grinding the tissue in a meat grinder and then pressing the juice out through cheesecloth. The samples of juice were kept in a refrigerator until ready for use. The pH values were first determined with the quinhydrone electrode and then N/20 NaOH (1 cc at first and 2 cc later) was added until a value above 8.3, the turning point for phenolphthalein, was reached. The pH values calculated from the successive potential differences thus obtained were plotted against the volume of NaOH required to produce them. From these curves the volume of alkali required to bring the reaction to pH 8.3 was determined and taken to represent the titratable acidity of the sample.

The results thus obtained are presented in table 4. It is evident from the values shown that in the first two experiments the pH, as well as the titratable acidity, of the healthy tops checked closely. This was also true for the diseased tops and roots. The chief disagreement was in the titratable acidity of the healthy roots in the second experiment. Since the second experiment was conducted on the day following the first, the plants were approximately the same age. The last experiment, however, was performed 12 days after the second, and the tops, which had been cut on October 4, were quite young. These younger tops, in the case of both the healthy and the diseased, had a higher pH value and a lower titratable acidity than the corresponding older ones used in the first two experiments. The pH values of the healthy and the diseased roots differed little from those in the other experiments. The titratable acidity of the diseased roots differed considerably, that in the third experiment requiring nearly 10 cc more alkali than did those in the two previous experiments. It is difficult to evaluate the 25.3 cc of NaOH used for the healthy roots. Since it agrees so closely with the comparable figure for the second experiment, it seems probable that the 22.8 cc in the first experiment is the variable one and that there was no appreciable change in the healthy roots because of the new growth. A comparison of the healthy and diseased tops shows that the former had slightly higher pH values than the latter. The differences are so small, however, that they probably are not significant. On the other hand, the diseased tops in each experiment had a higher titratable acidity than

the healthy tops, although that for the third experiment was identical with that for the healthy tops in the first experiment. There was almost no difference between the pH values of the healthy and diseased roots, but the healthy roots had a higher titratable acidity in the first two experiments.

TABLE 4.—*The pH and titratable acidity*[1] *of healthy and diseased alfalfa tops and roots, 1931*

Date	Tops				Roots			
	Healthy		Diseased		Healthy		Diseased	
	pH	N/20 NaOH	pH	N/20 NaOH	pH	N/20 NaOH	pH	N/20 NaOH
		Cc		*Cc*		*Cc*		*Cc*
Oct. 1	6.10	16.0	6.05	17.3	5.80	22.8	5.80	20.6
Oct. 2	6.15	16.3	6.00	17.0	6.05	26.0	5.85	20.4
Oct. 14	6.45	13.0	6.20	16.0	5.80	25.3	5.90	30.0

[1] Titratable acidity is expressed as cubic centimeters of N/20 NaOH required to bring 25 cc of juice to pH 8.3.

In general, it may be concluded that the diseased tops had a slightly higher hydrogen-ion concentration and a higher titratable acidity than did the healthy tops, that there was practically no difference in the pH values of the roots, and that the diseased roots had a lower titratable acidity than the healthy ones except in the last experiment where the reverse was true. It is also shown that the increased vegetative vigor of the tops in the third experiment resulted in decreased acidity, the greatest difference being in the healthy tops, which were growing most vigorously. Since the disease is most evident in the roots, at least in the earlier stages, and the pH value of the diseased and healthy roots is almost identical, it seems evident that the disease does not affect the pH value of the plant, the slight difference in the pH value of the tops being attributed to the difference in the stage of their development, that is, the less vigorous growth of the diseased tops is reflected in the lower pH. Whether the slight variation in the titratable acidity of the diseased and healthy roots was due to a difference in vigor or whether it signified the presence of a diseased condition is not known. Since such a great variation existed in the titratable acidity of the diseased roots between the first two experiments and the last, seemingly owing to a change in the stage of growth of the tops, it appears probable that the condition of growth also was responsible for the smaller differences between the diseased and healthy roots.

ASH CONTENT OF HEALTHY AND DISEASED PLANTS

On July 10, 1931, healthy and diseased alfalfa plants were taken from the same place in a 4-year-old stand, the tops were removed, washed in distilled water, and wiped dry. The roots were washed in tap water and distilled water. After being cleaned, the tops and roots were divided into two samples, placed in paper bags, and dried in an oven at 75° C. The dried samples were then ground fine and ashed, and the ash was calculated as percentage of dry matter. A second

set of samples was obtained on July 24, 1931, and treated in the same manner, making four samples for each date. The data obtained (table 5) show that the ash content of both the healthy tops and roots is considerably lower than that of the diseased. In fact, the healthy tops and roots had an average of only 88 and 67 percent as much ash as the diseased tops and roots, respectively.

TABLE 5.—*Ash content of healthy and diseased alfalfa tops and roots, 1931* [1]

Date of collection	Ash content [2] of—			
	Tops		Roots	
	Healthy	Diseased	Healthy	Diseased
	Percent	*Percent*	*Percent*	*Percent*
July 10	10.10	11.13	4.27	5.85
July 24	7.73	9.10	3.43	5.65

[1] The writer is indebted to Dr. A. R. C. Haas, of the California Agricultural Experiment Station for these determinations.
[2] Expressed as percentage of dry matter.

STARCHES AND SUGARS

The differences in the rate of transpiration and in ash between diseased and healthy plants show that there is an upset in the normal physiology of the alfalfa plants affected with dwarf. Further evidence of this is supplied by a study of the carbohydrate relations of the diseased plants. Although an analytical study was not made, a few observations, especially on the starch content of the affected roots, show clearly that a marked change in the carbohydrate content of the roots takes place as the disease progresses. Many diseased and healthy roots have been sectioned, stained with iodine, and examined. These studies show that in the early stages of the disease the affected roots are gorged with starch just as are the healthy roots. As the disease progresses the starch becomes less abundant, and by the time the disease is well advanced there is little or no starch left. The only observation made on the sugars of the diseased root is that some reducing sugar is present in roots from which the starch has entirely disappeared.

SUMMARY

Alfalfa roots affected with dwarf have more or less yellow color in the wood, owing largely to the presence of gum in the vessels. In the late stages of the disease there also is present a yellow stain, which diffuses into the surrounding tissues. The gum, which is similar in character to wound gum, is limited almost entirely to the vessels in the outer xylem, the other tissues being largely unaffected. In the early stages of the disease the gum is limited to a few vessels in one or more bundles in the upper part of the taproot or crown, but before the plant is killed the whole root system is involved. Gum is found in the green stems, if at all, only in the basal 1 or 2 inches. Some of the gum evidently is forced from adjacent cells into the ducts, where it appears in the form of globules or thin sheets along the inner surface of the

walls. Eventually many of the ducts become completely filled with gum and are rendered functionless.

Many bacterialike bodies are present in some of the vessels. Microchemical tests indicate that they are chemically very similar to the gum in which they are embedded and unlike bacteria in several respects.

The water content of the tops of healthy plants was slightly higher than that of diseased tops; the reverse was true of the roots. Whether this difference is sufficiently great to be significant is questionable.

Healthy alfalfa plants transpired about 1.6 times as fast as did diseased plants.

Water could be pulled through segments of healthy roots about 1.6 times as fast as through diseased roots of approximately equal diameter, indicating a close correlation between transpiration and the rate at which water could pass through the root segments.

The diseased tops had a slightly higher hydrogen-ion concentration and a higher titratable acidity than did the healthy tops, there was no difference in the pH value of the roots, and the diseased roots had a lower titratable acidity than the healthy roots except in one experiment. It seems probable that the differences in acidity between the healthy and diseased plants were due to a difference in growth.

Roots and tops affected with dwarf had a higher ash content than did healthy plants grown under similar conditions.

Qualitative tests showed that the starch in diseased roots gradually diminishes and that it disappears altogether just before the plant dies.

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INHERITANCE OF RESISTANCE TO PYTHIUM ROOT ROT IN SORGHUM[1]

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INTRODUCTION

A destructive root rot disease that attacks milo, a grain sorghum (*Sorghum vulgare* Pers.), was observed in 1926 on the Garden City Substation of the Kansas Agricultural Experiment Station at Garden City, Kans.[2] It was determined about 5 years later that the disease is caused by a pathogenic, soil-borne organism.[3] Recently this organism was identified as *Pythium arrhenomanes* Drechs.[4] The roots of diseased plants are decayed and discolored, and these symptoms usually spread to the crown and stalk. In the field the affected plants generally die and dry up before heads can be formed, and in the greenhouse they usually are killed before they are 8 or 10 inches tall (fig. 1, *A*).

Milo cannot be produced on badly infested soil. Since 1927 this so-called milo disease has been observed on milo in Texas, New Mexico, Oklahoma, and California, as well as in Kansas at localities other than those mentioned herein. Once the disease becomes established, it often spreads rapidly throughout a field. It usually occurs where milo is grown continuously or in alternate years.

The pythium root rot of sorghums has been found only on milo and darso and on hybrids involving one or both of these types. Selections of resistant strains of milo and its hybrids are showing marked resistance in the field and greenhouse.[5] [6] The distinct host reactions shown in field and greenhouse tests for resistance to pythium root rot appeared to justify studies of the inheritance of resistance to the disease, the results of which are reported here. The data presented include two independent sets of greenhouse experiments begun concurrently at Manhattan, Kans., and at Arlington Experiment Farm, Arlington, Va. (near Washington, D. C.), together with supplementary results obtained in the field at Garden City, Kans.

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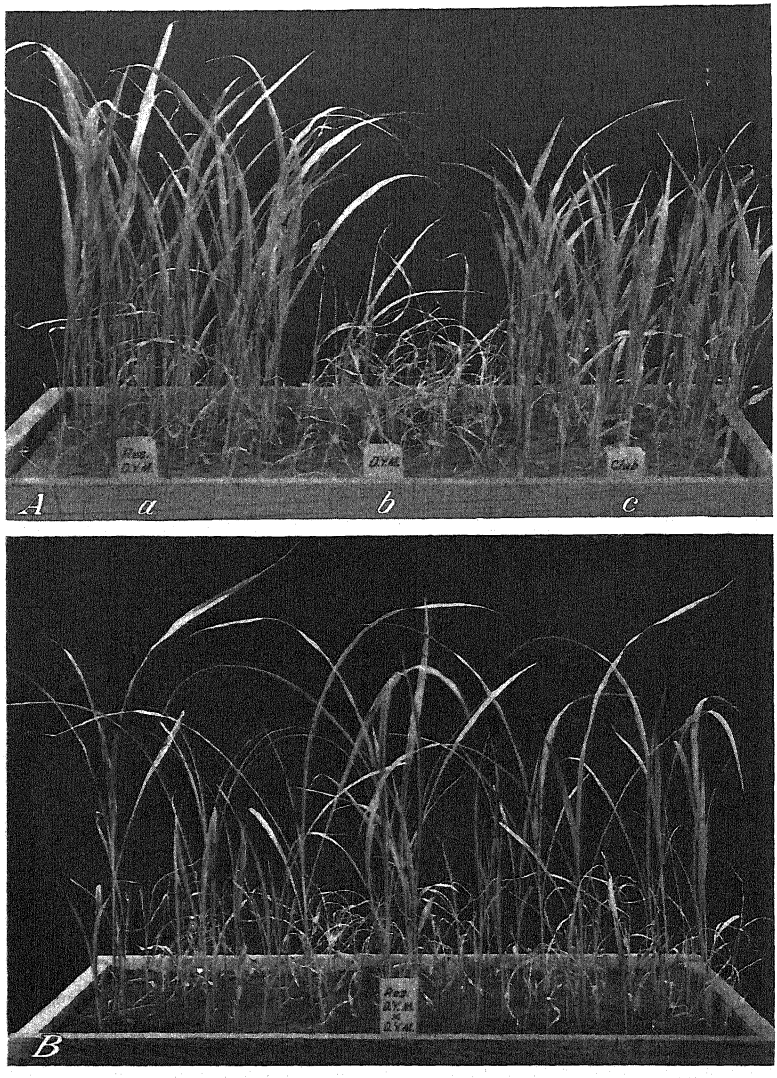


FIGURE 1.—Reaction to the milo disease of sorghum grown in infested soil in the greenhouse at Manhattan, Kans. *A*, Three parent varieties: *a*, Resistant Dwarf Yellow milo; *b*, Dwarf Yellow milo (susceptible); *c*, Club kafir (resistant). *B*, F_2 population of the cross Resistant Dwarf Yellow milo × Dwarf Yellow milo (susceptible). The tall, healthy plants are resistant segregates; the short, dead plants are susceptible to *Pythium*.

MATERIAL AND METHODS

Sorghum varieties were chosen for crossing on the basis of their reaction in nursery tests at Garden City, Kans., on infested soil. A comparison of resistant and susceptible varieties is shown in figure 1, *A*.

The susceptible varieties involved in the 12 crosses studied were Dwarf Yellow milo (C. I.[7] 332), Day milo (C. I. 959) (dwarf early

[7] C. I. refers to accession number of the Division of Cereal Crops and Diseases, formerly Office of Cereal Investigations.

type), Beaver (C. I. 871), Custer (C. I. 919), kafir × milo (No. 27–317, C. I. 963), and Early kalo (C. I. 1009). The last four were all derived from kafir × milo hybrids. The resistant varieties included Club kafir (C. I. 901), Western Blackhull kafir (C. I. 906), White kaoliang (C. I. 792), Black Amber sorgo (F. C.[8] 7038), and Resistant Dwarf Yellow[9] milo. The last-named variety was developed by F. A. Wagner from a single Dwarf Yellow milo plant that survived the disease in a severely infested plot at Garden City.

The crosses tested at Manhattan were made in the greenhouse by C. A. Wismer in the winter of 1932–33 and by D. H. Bowman and A. E. Lowe in the winter of 1933–34. The F_1 plants were grown on noninfested soil in the nursery at Manhattan in 1933 or 1934. The five crosses tested at Arlington Farm were made by John H. Martin. The F_1 plants were grown in noninfested soil in the greenhouse.

The infested soil for greenhouse experiments, which was supplied by F. A. Wagner, superintendent of the Garden City Substation, was taken from portions of the field in which the milo disease experiments had been conducted for several years, and was known to be infested. Flats were filled with the soil to a depth of about 2½ inches. Seed of the sorghum hybrids and the parental varieties was planted in rows across the flats, 8 rows to the flat at Manhattan, Kans., and 10 rows at Arlington Farm. In most of the experiments at Manhattan and in all at Arlington Farm the parental varieties and the hybrids involving them were planted in the same flat. With a few exceptions the parental varieties occupied the two outside rows of each flat. At Manhattan, three plantings were made during the winter of 1933–34 of seed from F_1 plants grown in the nursery in 1933. Each flat was thus replanted twice after final notes had been taken on the susceptibility of the previous crop and the plants had been removed. Remnant seed from the greenhouse experiment of 1933–34 was planted by F. A. Wagner on diseased soil at Garden City to supply additional data, under field conditions, on the inheritance of resistance in the F_2 generation.

In November 1934, seed was planted in the greenhouse from the F_1 plants grown in the nursery at Manhattan that season, from the additional crosses made during the previous winter. After notes on resistance had been taken the flats were replanted twice with seed from F_2 plants of the same crosses that had been tested in the greenhouse during the winter of 1933–34. The F_2 material for this third-generation study, however, had been grown on clean land in the nursery at Manhattan in 1934. Because of drought, chinch bugs, and killing frosts before maturity, seed from both F_1 and F_2 plants grown in the nursery at Manhattan in 1934 was decidedly limited and of low viability.

Three successive plantings also were made in the greenhouse at Arlington Farm from December 1933 to April 1934. By thus replanting the flats, greenhouse space could be used very efficiently for testing sorghums for resistance to pythium root rot.

At Manhattan, the seed was treated either with copper carbonate or Semesan Jr. to control seed rots before planting in the flats. The latter dust fungicide appeared to be preferable. These seed treat-

[8] F. C. refers to accession number of the Division of Forage Crops and Diseases.

[9] Since the submittal of this manuscript for publication the name of this variety has been changed to "Finney."

ments did not seem to affect the development of pythium root rot in these tests or in previous experiments by others.[10] The seed at Arlington Farm was not treated before planting, and poor stands resulted from seed rotting and from a seedling blight caused by a



FIGURE 2.—Plants from the F_2 population of the cross Resistant Dwarf Yellow milo × Dwarf Yellow milo (susceptible), grown in infested soil in the greenhouse at Manhattan: *A*, Resistant (20 plants); *B*, intermediate (15 plants); and *C*, susceptible (60 plants).

species of *Penicillium*. Susceptibility to *Pythium* and to *Penicillium* showed no association.

Symptoms of pythium root rot appeared about a month after planting and developed rapidly, so that final notes could be taken 2 to 4 weeks later. The first indication of infection in the seedling stage was the limp, ashy-gray, or seemingly scalded condition of the leaves.

[10] MELCHERS, L. E., and PARKER, J. H. CONTROL MILO DISEASE. Cappers Farmer 45: 27. 1934.

A few days later an orange tinge could be detected around the outer margin of the leaf. This color advanced over the entire plant and gradually changed to brown as the leaves dried. The youngest leaf was the last to discolor and die. Finally, the dead plants became light brown and remained upright for some time, not falling over as do plants attacked by some of the common molds.

The greenhouse temperature in the period from 1933–34 usually varied between 75° and 85° F. at Manhattan and between 70° and 80° at Arlington Farm. Duplicate plantings of nine grain sorghum varieties at two greenhouse temperature ranges, 65° to 70° and 75° to 80°, were made at Manhattan in 1934–35. This 10-degree difference in average temperature did not affect the expression of disease reaction in the varieties. Similar conclusions have been reported by others.[11]

The methods of recording the data were somewhat different in the two sets of experiments, although at both stations final counts of resistant and susceptible plants usually were made when all plants of the susceptible parent had died. At Manhattan, the plants were divided into three classes: (1) Dead (susceptible), (2) apparently diseased but not dead (intermediate), and (3) healthy (resistant) (fig. 2). Plants in class 1 could be determined easily by observation, but surviving plants were examined for general thriftiness, dead roots, and red discoloration in the central cylinder of infected roots. Plants showing lack of thriftiness and dead or discolored roots were classed as susceptible. Questionable plants were further examined by splitting the crowns. The presence of a deep reddish brown in the crown or in the subcrown internode was considered an indication that infection had taken place, as this condition was not found in healthy plants. Diseased plants were indicated also by a yellowing of the leaves. Roots arising from a discolored portion of the crown were almost always dead or dying and discolored, but any roots arising above the discolored portion of the crown often permitted the plants to survive until all of the roots became diseased. The red discoloration first appeared in the central cylinder of the root and spread later to the entire root. The dead and intermediate plants were all regarded as susceptible and are classed together in the tables that follow. Some of the roots of resistant plants showed a pinkish color, but the rest of the plant appeared healthy.

GENETIC RESULTS

THE F_1 GENERATION

One cross, Beaver milo×Resistant Dwarf Yellow milo, was tested for resistance in the F_1 generation at Arlington Farm. Only three plants emerged from the 10 seeds planted on November 11, 1933. These plants at first showed considerable resistance to *Pythium* in comparison with the susceptible Beaver parent and were still alive on December 25, when six of the eight Beaver plants were dead. By January 22, 1934, however, when the remaining Beaver plants were dead, the hybrid plants also had died. At that time the 12 plants of the Resistant Dwarf Yellow milo were still healthy. This indicated that resistance in the F_1 generation was intermediate between the

[11] ELLIOTT, C., MELCHERS, L. E., LEFEBVRE, C. L., and WAGNER, F. A. See footnote 4

parents, with a tendency toward a dominance of susceptibility. During the period of early growth it seemed that the F_1 hybrids more nearly approached the behavior of the resistant parent, but this apparent reaction did not continue.

THE F_2 GENERATION

RESULTS AT MANHATTAN, KANS.

The segregations for disease resistance in the F_2 hybrids grown at Manhattan in the seasons 1933–34 and 1934–35 are shown in table 1. The numbers of plants listed for 1933–34 are the totals for the three plantings that year. The cross Club×Resistant Dwarf Yellow milo produced all resistant plants, as might be expected from the resistance of both parents. Eight plants in a total of 392 of the Resistant Dwarf Yellow milo were classed as susceptible, but the causes of the apparent disease attack were not determined.

TABLE 1.—*Segregation for resistance to Pythium in the F_2 generation of sorghum crosses in the greenhouse at Manhattan, Kans., and at the Arlington Experiment Farm, Arlington, Va., and in the nursery at Garden City, Kans.*

Variety or F_2 hybrid	Observed			Calculated		Deviation and probable error	Deviation / Probable error
	Total	Susceptible	Resistant	Susceptible	Resistant		
	Number	*Number*	*Number*	*Number*	*Number*	*Number*	
GREENHOUSE, MANHATTAN, KANS., 1933-34							
Dwarf Yellow milo (susceptible)	426	425	1				
Resistant Dwarf Yellow milo (resistant)	392	8	384				
Club kafir (resistant)	390	0	390				
Club kafir×Resistant Dwarf Yellow milo	1,205	0	1,205				
Club kafir×Dwarf Yellow milo	1,006	728	278	754.5	251.5	26.5±9.26	2.86
Dwarf Yellow milo×Resistant Dwarf Yellow milo and reciprocal	2,402	1,732	670	1,801.5	600.5	69.5±14.31	4.86
GREENHOUSE, MANHATTAN, KANS., 1934-35							
Beaver (susceptible)	51	51	0				
Custer (susceptible)	100	100	0				
Kafir×milo, No. 27-317 (susceptible)	92	92	0				
Dwarf Yellow milo (susceptible)	168	168	0				
Resistant Dwarf Yellow milo	564	1	563				
Resistant Dwarf Yellow milo×(kafir×milo, No. 27-317)	475	6	469				
Resistant Dwarf Yellow milo×Custer	218	139	79	163.5	54.5	24.5±4.25	5.76
Resistant Dwarf Yellow milo×Beaver	1,010	727	283	757.5	252.5	30.5±9.28	3.28
NURSERY, GARDEN CITY, KANS., 1934							
Club kafir×Dwarf Yellow milo	149	107	42	111.8	37.2	4.8±3.56	1.33
Resistant Dwarf Yellow milo×Dwarf Yellow milo	736	542	194	552	184	10.0±8.02	1.24
GREENHOUSE, ARLINGTON FARM, 1933-34							
Beaver (susceptible)	12	12	0				
Beaver×White kaoliang	221	68	153	55.2	165.8	12.8±4.34	2.94
White kaoliang (resistant)	22	0	22				
Day milo (susceptible)	18	18	0				
Western Blackhull kafir×Day milo	368	120	248	92	276	28.0±4.85	5.77
Western Blackhull kafir (resistant)	34	0	34				
Club kafir (resistant)	25	0	25				
Club kafir×Day milo	346	69	277	86.5	259.5	17.5±5.43	3.22
Day milo (susceptible)	17	16	1				
Black Amber sorgo (resistant)	88	0	88				
Day milo×Black Amber sorgo	892	138	754	223	669	85.0±8.72	9.75
Day milo (susceptible)	22	13	9				

The Club kafir × Dwarf Yellow milo cross segregated according to a monohybrid ratio within the limits of random error. The cross Dwarf Yellow milo × Resistant Dwarf Yellow milo and the reciprocal of this cross (fig. 1, *B*) deviated somewhat from the 3 : 1 ratio, owing to an excess of resistant plants. The cross and reciprocal were each grown in three plantings, and in four of the six tests the observed number of resistant plants exceeded the calculated number. Although in five of the six tests the deviation from a calculated 3 : 1 ratio lay within three times the probable error, when the data are combined, as in table 1, the deviation becomes statistically significant.

At Manhattan in 1934–35 the cross Resistant Dwarf Yellow milo × (kafir × milo, No. 27–317) produced only six susceptible plants, although the latter parental variety was fully susceptible. The most plausible explanation of this is the probable difference in disease reaction between the kafir × milo plant used as the parent in the cross and the bulk variety that was tested for disease resistance. In the crosses Resistant Dwarf Yellow milo × Custer and Resistant Dwarf Yellow milo × Beaver, the number of resistant plants exceeded the calculated number by somewhat more than three times the probable error. Additional material, supposedly of the cross Resistant Dwarf milo × Custer, proved to be almost entirely resistant. This may have been due to an error in making the hybrids or to the use of a resistant plant from the susceptible Custer variety as a parent.

Seed of the cross Resistant Dwarf Yellow milo × Early kalo (susceptible) was planted at Manhattan in 1934–35, but because of poor seed only six plants emerged, and the data are not shown.

Results at Garden City, Kans.

The data obtained from two crosses grown in the F_2 generation in the Garden City disease nursery in 1934 are shown in table 1. The reactions noted were based upon an external examination of the plants in the field on October 1, 1934. In both crosses the deviation from a 3 : 1 ratio only slightly exceeded the probable error. These results, together with those obtained from the same crosses in the greenhouse at Manhattan in 1933–34, strongly suggest a single major genetic factor difference for the inheritance of resistance to *Pythium*.

The cross Club × Resistant Dwarf Yellow milo and its reciprocal also were grown at Garden City in 1934 but produced resistant plants only. This result, substantiating the behavior of this cross at Manhattan in 1933–34, indicates that the same factor for resistance is found in both parents.

Results at the Arlington Experiment Farm

Some difficulty was encountered at Arlington Farm in classifying the plants, owing to reddish discoloration of the roots possibly from causes other than *Pythium* infection. Consequently, the plants were classified at several intervals on the basis of whether they were dead or alive. Final notes usually were taken when all the plants of the susceptible parent of any particular cross were dead. At that time hybrid plants showed all gradations of development, from fully healthy to dead and dried. In two experiments at Arlington Farm,

not all the plants of the susceptible variety were dead when the final notes were taken. The condition of the susceptible parent was regarded as the most satisfactory standard for determining complete susceptibility among the hybrids. The data from Manhattan and Arlington Farm, although recorded differently, should lead to the same genetic conclusions because the difference lies merely in grouping the intermediate (or apparently heterozygous) plants with either the fully susceptible or the resistant segregates.

The data from the four crosses tested in the F_2 generation at Arlington Farm in 1933–34 are shown in table 1. The plants classed as susceptible included only those dead at the time final notes were taken. The Beaver × White kaoliang cross segregated according to



FIGURE 3.—Segregating F_2 row of the cross Western Blackhull kafir × Day milo, grown in the same flat of *Pythium*-infested soil as the parents, shown in figure 4, at the Arlington Experiment Farm.

a simple monohybrid ratio. In the Club kafir × Day milo cross the deviation from a 3:1 segregation was slightly greater than three times the probable error. In this experiment, however, one of the 17 Day milo plants was still alive when the final notes were taken. The Western Blackhull kafir × Day milo cross showed a deviation from the calculated ratio amounting to 5.77 times the probable error. The deviation was much greater in the first planting than in the second, where the ratio was very close to 3:1 (fig. 3). Both the resistant and the susceptible parent of this cross are shown in figure 4.

The test of the Day milo × Black Amber sorgo cross was of necessity discontinued before all plants of the susceptible parent, Day milo, were dead, although all plants of that variety were badly wilted and stunted. Much less than a fourth of the hybrid plants were dead at that time and those surviving showed widely varying degrees of injury.

The data as presented in table 1 indicate a wide deviation from a simple monohybrid segregation. Only 13 of the 22 Day milo parent plants in the same soil flats were dead at the time final notes were taken. If the number of dead hybrid plants is adjusted in accordance with the proportion of dead plants of the Day milo parent, the results are in close agreement with a simple monohybrid ratio.

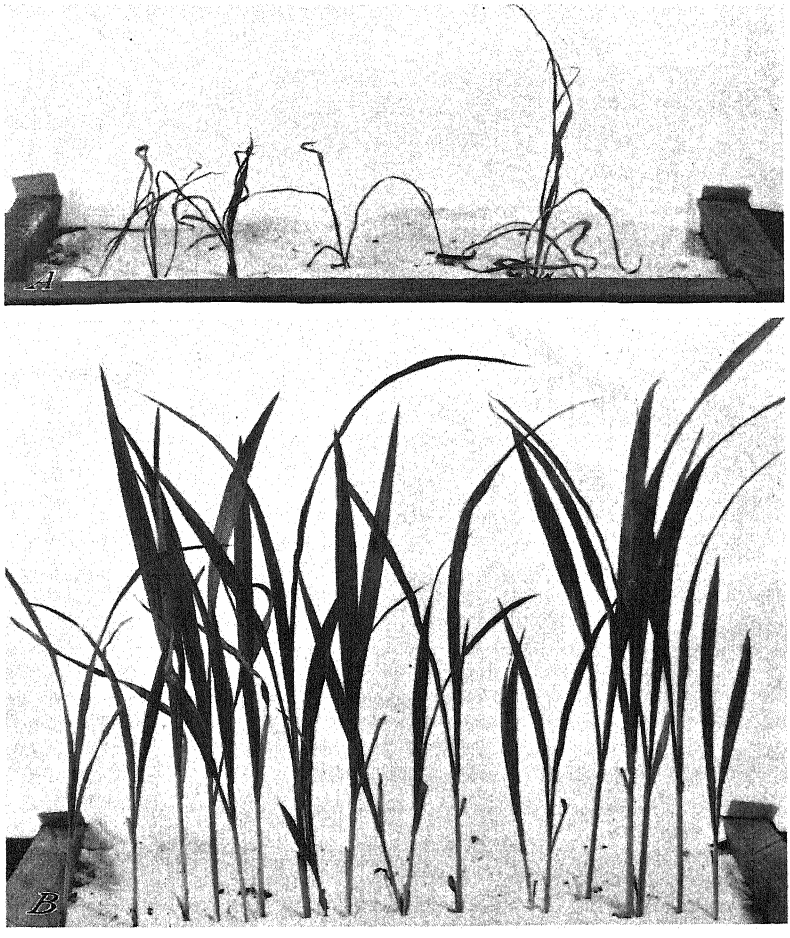


FIGURE 4.—Resistant and susceptible sorghum plants grown in the same flat of *Pythium*-infested soil at the Arlington Experiment Farm. *A*, Day milo (susceptible); *B*, Western Blackhull kafir (resistant).

THE F_3 GENERATION

The behavior of 19 F_3 lines of two crosses in the greenhouse at Manhattan, Kans., in 1934–35, is shown in table 2. Nine additional lines produced so few plants, owing to nonviable seed, that their behavior could not be determined. Considering the 19 lines of the two crosses together, 7 were classed as resistant and 12 as susceptible or segregating. The calculated numbers are 4.75 and 14.25, on the basis

of a simple monohybrid ratio, and are in rather close agreement with expected results. Each of the five segregating lines indicated a statistically significant monohybrid ratio, as did the combined results from the segregating lines of each cross. These data verified the conclusions pointing to simple inheritance that were based on data obtained in the F_2 generation of these crosses.

TABLE 2.—*Reaction to Pythium in the F_3 generation of 19 lines in two sorghum crosses grown in the greenhouse at Manhattan, Kans., 1934–35*

Cross and reaction of line	Total plants	Susceptible plants	Resistant plants	Deviation from 3:1 ratio	Deviation / Probable error
Club kafir (resistant)×Dwarf Yellow milo (susceptible):	*Number*	*Number*	*Number*		
Resistant	204	4	200		
Do	264	1	263		
Do	192	5	187		
Susceptible	41	41	0		
Do	192	192	0		
Do	252	252	0		
Do	200	200	0		
Segregating	28	20	8	1.00±1.54	0.65
Do	89	72	17	5.25±2.75	1.91
Total segregating	117	92	25	4.25±3.16	1.35
Dwarf Yellow milo (susceptible)×Resistant Dwarf Yellow milo and reciprocal:					
Resistant	78	0	78		
Do	38	0	38		
Do	31	0	31		
Do	53	0	53		
Susceptible	36	36	0		
Do	41	41	0		
Do	32	32	0		
Segregating	28	22	6	1.00±1.54	.65
Do	49	42	7	5.25±2.04	2.57
Do	68	50	18	1.00±2.41	.41
Total segregating	145	114	31	5.25±3.52	1.49

With regard to all generations of all crosses, it appears that susceptibility is partly dominant, and the segregations support the conclusion that a single major genetic factor pair largely determines the susceptibility of sorghums to pythium root rot.

COLEOPTILE COLOR AND PYTHIUM RESISTANCE

One planting of the Beaver×White kaoliang cross at Arlington Farm was classified for seedling or coleoptile color. The red coleoptile character present in the Beaver parent has been shown by Reed [12] and others to be inherited as a simple dominant character in many sorghum crosses. Small stakes were placed beside each plant having a green coleoptile, and the coleoptile color was recorded when counts of dead plants were made. The data are shown in table 3.

In this experiment the 132 plants segregated in exactly a 3:1 ratio for resistant and susceptible plants. There was a deviation of only 2 from the calculated 3:1 ratio for plants having red or green coleoptiles. When the segregations for the two characters are combined, it is seen that the number of plants in each class is very

[12] REED, G. M. A NEW METHOD OF PRODUCTION AND DETECTING SORGHUM HYBRIDS. Jour. Heredity 21: 132-144, illus. 1930.

close to the expected 9:3:3:1 ratio on the basis of independent inheritance. It appears from these results that seedling, or coleoptile, color is inherited independently of resistance to pythium root rot.

TABLE 3.—*Segregation for coleoptile color and susceptibility to Pythium in the F_2 generation of the sorghum cross Beaver × White kaoliang, in the greenhouse at the Arlington Experiment Farm, 1933–34*

Type and condition of plants	F_2 hybrid plants					Parent plants	
	Observed	Calculated (9:3:3:1 ratio)	Deviation and probable error	Calculated (3:1 ratio)	Deviation and probable error	Beaver milo	White kaoliang
Red coleoptile:	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*
Alive	74	74.25	0.25±0.47			0	0
Dead	27	24.75	2.25± .37			4	0
Total	101			99	2±3.36	4	0
Green coleoptile:							
Alive	25	24.75	.25±.37			0	7
Dead	6	8.25	2.25±.23			0	0
Total	31			33	2±3.36	0	7
Total alive	99			99	0		
Total dead	33			33	0		
Grand total	132			132		4	7

RELATION OF HETEROSIS TO PYTHIUM RESISTANCE

All the crosses tested at Manhattan, with the exception of the Resistant Dwarf Yellow milo × Dwarf Yellow milo (susceptible), showed heterosis, or hybrid vigor, in the F_1 generation, manifested chiefly by an increase in height of from 9 to 32 inches over that of the taller parent. These resistant and susceptible strains of Dwarf Yellow milo differ in resistance to *Pythium*, but they are very similar in appearance. Past experiments with sorghum hybrids have demonstrated that increased vigor is carried into the second generation.

Segregation for pythium resistance was substantially the same in Resistant Dwarf Yellow milo × Dwarf Yellow milo (susceptible) as in the other crosses in which heterosis was apparent. It would appear, therefore, that heterosis per se did not alter the inheritance of resistance.

SUMMARY

Progenies of crosses between sorghum varieties resistant or susceptible to pythium root rot (or the "milo disease"), caused by *Pythium arrhenomanes* Drechs., were studied in infested soil in the greenhouse at Manhattan, Kans., and the Arlington Experiment Farm, Arlington, Va., and in the field at the Garden City Substation of the Kansas Agricultural Experiment Station.

The data indicate that susceptibility to the disease is partly dominant. Reaction to the disease is determined by a single major factor difference.

Susceptibility is inherited independently of coleoptile (seedling) color and of hybrid vigor of the progenies.

OBSERVATIONS OF PATHOGENIC AND ANTIGENIC EFFECTS OF BRUCELLA ABORTUS, UNITED STATES BUREAU OF ANIMAL INDUSTRY STRAIN 19 [1]

By C. M. HARING, *head of division*, and J. TRAUM, *veterinarian, Division of Veterinary Science, California Agricultural Experiment Station* [2]

INTRODUCTION

Investigators at the United States Bureau of Animal Industry Experiment Station, Bethesda, Md., have reported encouraging results from the use of subcutaneous injections of living *Brucella abortus* strains of moderate or low virulence to immunize cattle against infectious abortion (Bang's disease). The first publication regarding this work at Bethesda was made by Buck (*1*)[3] in 1930, and subsequent reports have been issued by Mohler (*11*), and by Cotton (*3*). The status of these experiments in 1933 was described by Cotton and Buck (*4*), and the technical procedures and results up to 1934 were published in detail by Cotton, Buck, and Smith (*5*, *6*, *7*, *8*).

One of the cultures reported by these investigators to yield promising results in immunizing heifers against *Brucella* infection was designated by them as strain 19. They claim that this strain is fairly constant in pathogenic power as indicated by repeated tests on guinea pigs, and that it is sufficiently low in its virulence for nonlactating and nonpregnant dairy cattle to be relatively free from the objectionable feature of becoming permanently implanted in the udder following its use as vaccine.

In 1932 transplants of strain 19 were obtained by the writers from the Chief of the Bureau of Animal Industry. The following report is a summary to May 1937 on observations of its effects on guinea pigs and cattle at the California Agricultural Experiment Station. Photostatic copies of the life history for each vaccinated and control animal subjected to test exposure may be had by official investigators on application to the writers of this article.

METHODS

The methods used at the California station have conformed in general to those described in the publications by Cotton, Buck, and Smith. In transmitting cultures of strain 19 to the writers, the Chief of the Bureau of Animal Industry described these methods in a letter dated October 10, 1932, which included the following statements:

> You are advised that potato-agar slants of *Brucella abortus* strain 19, which is of moderate virulence and has given encouraging results at Bethesda, are sent you under separate cover.
>
> The method of preparing the vaccine from this culture is as follows: The growth from 48- to 72-hour potato-agar slants is washed off with 0.85 percent salt

[1] Received for publication Sept. 16, 1936; issued August 1937.
[2] Acknowledgment is made to Mrs. Phyllis A. Zweigart, agent, U. S. Bureau of Animal Industry, for the bacteriologic procedures in this work. That bureau has also furnished laboratory and clerical assistance. The Works Progress Administration furnished assistants to care for experimental animals.
[3] References are made by number (italic) to Literature Cited, p. 128.

solution and diluted to a density of 10 times tube 1 of the McFarland's nephelometer. This is used shortly after it is prepared, usually the same day.

The animals are usually vaccinated at least 2 months before breeding and it would perhaps be better if this time were extended until agglutination reactions induced have subsided to a considerable degree. Of course, when calves are vaccinated the period is much longer.

Except for calves, 10 or 20 cc of the vaccine have been given. Seemingly, the dose may vary within considerable limits without appreciably affecting results. If more than 10 cc of vaccine are given the amount is divided between two points. Injections are usually made in front of the shoulder.

We endeavor to expose the animals when they are from 2 to 5 months pregnant. A suspension of a virulent *Brucella abortus* culture is used in making the exposures, which are by the conjunctival method. Our experiments have indicated that this method may be made to be overwhelming, therefore, we have found it important to keep it within reasonable bounds by adjusting the dosage.

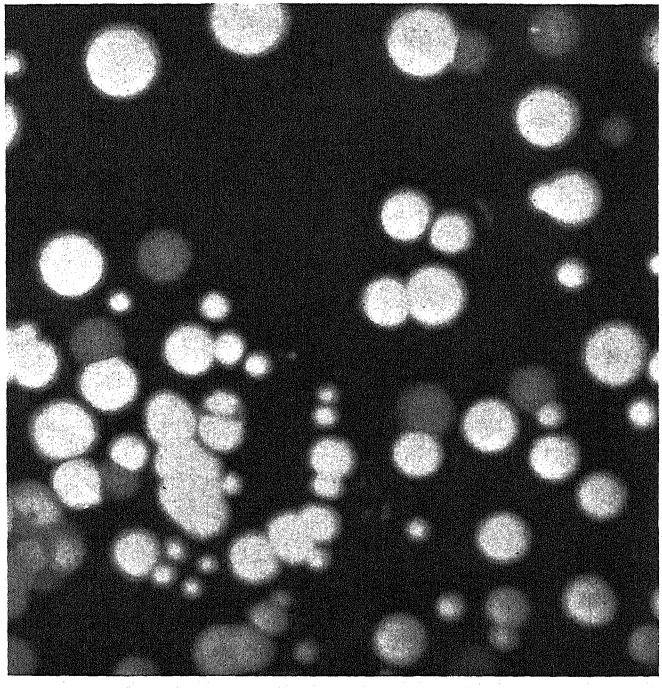
For a few months after the strain was received it was propagated on potato-agar slants, but since January 1933 Liebig's beef infusion agar, containing 2 percent of glycerin and 1 percent of glucose has been used. No change in cultural or pathogenic characteristics has been observed, and the strain has continued to conform to the descriptions published by Cotton, Buck, and Smith. When it is plated in agar, both S and R type colonies develop. In plate 1 are shown R- and S-type colonies which developed in glucose glycerin agar after being planted with a 28-day plain broth culture.

For the last 2 years strain 19 has been used at this laboratory for preparing antigens for the tube and plate methods of agglutination testing. About 80,000 tests of blood serums and whey have been made, and as yet no trouble has been experienced from self-agglutinations or other unsatisfactory effects that are supposed to be caused by the presence of R types in antigen. The precaution has been taken to occasionally renew the stock cultures from which the antigen is made by propagating from single S-type colonies.

For the isolation of *Br. abortus* from the experimental animals, the methods described by Henry, Traum, and Haring (*10*) have been followed, with the exception of the hemoculture technique which deserves special mention in view of the fact that several investigators, including the group at Bethesda, have reported difficulty in isolating *Br. abortus* from the blood stream of cattle.

Hemocultures were at first made according to the recommendations of Soule (*13*), but this proved cumbersome, laborious, and expensive, and yielded a high percentage of contaminations which rendered the method unsatisfactory. Fair success was obtained by a modification of the technique used by Shaw (*12*), which consisted in letting about 5 cc of blood run on a large cooked-blood agar slant, incubating for 6 or 7 days, stirring, and incubating again. These slants should be kept under observation for at least 2 weeks before discarding.

The best results in hemocultures were obtained by a modification of a method that has been described by Stewart, Eddie, Paxton, and Meyer (*14*). This modification consists in allowing about 5 cc of blood to run from the jugular vein into a sterile test tube in which has been placed 5 or 6 drops of a 1.6-percent solution of sodium citrate. The tube containing the citrated blood is heated in a water bath at 56° C. for 15 minutes, and 3 to 4 cc is poured over a large cooked-blood agar slant. A tube of not less than seven-eighths of an inch in diameter is used. This is incubated for 6 or 7 days in an atmosphere of 10-percent CO_2, after which the tube is tipped for a few seconds so that the blood will flow over the upper portions of the slants.



Colonies of Strain 19 on glucose-glycerin agar. This medium was seeded from a transplant in plain broth made to bring about dissociation. In this respect, Strain 19 behaves like most cultures of *Brucella abortus*. The R colonies appear white and the S colonies gray. × 15.

In this method the incubation is continued for at least 3 weeks, during which time it is examined at intervals of 3 or 4 days, and each time the tube is tipped so that the blood runs over the slant. When *Br. abortus* is present this results in an abundance of small colonies.

From a comparison of the results of blood cultured simultaneously by these different methods, it is evident that either the heating or the citrating of the blood, or both, is an important part of the technique of the latter procedure. The comparison is shown in table 1.

TABLE 1.—*Comparative results with untreated and citrated-heated blood cultures from cattle, made at various intervals after exposure to Brucella abortus*

Animal no.	Strain and method of exposure	Results from cultures of blood from jugular vein after indicated number of days following exposure [1]																							
		1		4		5		7		8		9		12		14		18		21		25		35	
		U	C	U	C	U	C	U	C	U	C	U	C	U	C	U	C	U	C	U	C	U	C	U	C
8	36 conjunctivally	−	−	−	−			co	co					−	+					−	−			−	−
9	do	−	−	−	−			−	co					−	+					−	−			−	−
10	do	−	−	−	−			−	−					−	+					−	+			−	−
1242	do	−	−			−	−			−	+					−	+			−	−			−	−
2183	do	−	−			−	−			−	+					−	−			−	−			−	−
2067	19 subcutaneously	−	−	−	−							−	−					−	+			−	−	−	−

[1] U indicates that fresh untreated blood was spread over the surface of cooked-blood agar slants; C indicates that citrated and heated blood was used in the same way. +=*Brucella* colonies observed; −=no *Brucella* colonies found; co=contaminated. Cultures were continued at fortnightly intervals for 100 days with negative results.

TESTS FOR VIRULENCE ON GUINEA PIGS

In addition to the use of direct cultures, approximately 200 guinea pigs have been inoculated with milk, uterine material, and other tissues of vaccinated cattle to test for the persistence of strain 19.

When this organism is present in the material inoculated it can usually be recovered by spleen culture on cooked-blood agar, provided the guinea pigs are killed about the sixth week after inoculation. Apparently the organisms of this strain tend to disappear from the guinea pig tissues soon after the sixth week. Macroscopic lesions in guinea pigs rarely result from the injection of milk or tissues containing only a few organisms of this strain. The agglutination titer is usually low. The absence of any apparent enlargement of the spleen or macroscopic change in the parenchyma of the spleen, liver, testes, or lymph nodes is in marked contrast to the definite lesions resulting from the inoculation of milk or tissues containing more virulent strains of *Br. abortus*.

When culture suspensions of a billion or more organisms of strain 19 are injected intra-abdominally a positive blood agglutination titer usually results and, occasionally, slight enlargement of the spleen and other slight lesions. A series of 18 guinea pigs were injected intra-abdominally with 0.2 to 0.3 cc of vaccine. Selections for autopsy from this group were made at varying intervals from 40 to 107 days. Positive cultures from the spleen were obtained in all cases up to 56 days. Two guinea pigs killed on the seventy-fourth day were negative. On the eighty-second day a third was positive and a fourth negative. Autopsies on the eighty-fourth and one hundred and

seventh day were negative. These results are in contrast to the findings in control guinea pigs inoculated with virulent strain U. C. V. 36 which yielded positive spleen cultures and definite lesions in every one of a series of 14 animals. The intervals of autopsy extended from the thirtieth to the one hundred and twelfth day.

WORK ON CATTLE WITH STRAIN 19

LONGEVITY IN BODIES OF VACCINATED CATTLE

In order to determine the longevity of strain 19 in the tissues of vaccinated cattle, and test for possible shedder conditions set up in them, a study was made of blood, subcutaneous and lymphnode tissues, uterine material, and milk. Hemocultures from the jugular veins of vaccinated calves and cows have shown that the organisms may circulate in the blood for at least 22 days following vaccination. The only instances in which the strain has been obtained from the milk or from uterine material are as follows:

Cow 2060 (table 2) discharged strain 19 in the uterine material and colostrum at the time of calving on the seventeenth day following her vaccination, but no evidence was found that she continued to shed the organisms in the milk or in the uterine material at two subsequent normal calvings.

TABLE 2.—*Results of vaccinating pregnant cows with Brucella abortus strain 19*

Group	Cow no.	Time pregnant when vaccinated	Gestation period	Description of calf	Results obtained with various materials tested for Br. abortus [1]					
					Guinea pig inoculations			Direct cultures		
					Uterine material	Colostrum	Calf tissues	Uterine material	Colostrum	Calf tissues
		Days	*Days*							
A, vaccinated while pregnant, but not otherwise exposed.	2187	28	280	Vigorous	−	−	------	------	------	------
	14	124	287	----do	−	−	------	−	−	------
	32	128	283	----do	−	−	------	−	−	------
	4	199	280	Normal (?)—found dead from exposure.	−	−	−	−	−	−
B, vaccinated while pregnant and 32 days later exposed conjunctivally to strain U. C. V. 36.	2060	253	270	Weak	+	−	+	+	+	+[2]
	2180	109	279	Vigorous	−	−	------	−	−	------
	2637	123	244	Premature; died next day.	+	−	−	+[3]	−	−
	35	128	253	Premature; lived 3 hours.	−	+	+	+	−	+[2]

[1] + = positive, − = negative.
[2] Cultures were characteristic of strain 19.
[3] Cultures were characteristic of strain 36.

Cow 35, vaccinated while pregnant 128 days, yielded strain 19 in a culture of blood from the jugular vein on the seventeenth day after vaccination, and again from the placenta and colostrum 125 days after vaccination.

Biopsy tests on a vaccinated bull have shown that the organism of strain 19 may remain alive for at least 24 days in the subcutaneous tissues and lymph nodes adjacent to the point of injection. Tests on

another bull, slaughtered on the one hundred and thirteenth day after vaccination, failed to show any living *Brucella* organisms, although direct cultures were made and several guinea pigs inoculated with a variety of tissues from various parts of the body, including the vicinity of the point of injection.

ASSOCIATION OF VACCINATED AND NONVACCINATED CATTLE

During the 3½ years that experiments with strain 19 have been in progress in Berkeley, it has been customary to keep the vaccinated cattle with approximately an equal number of nonvaccinated controls of about the same age. In addition, a few older cows have usually been in the same fields and corrals while in various stages of pregnancy. Most of the vaccinated and nonvaccinated animals have now passed through pregnancies, and inoculations of milk into guinea pigs, as well as frequent agglutination tests, have not indicated any transfer of infection from the vaccinated to the nonvaccinated cattle.

VACCINATION OF PREGNANT COWS

Cotton (*3*) and Buck (*2*) both advised against the vaccination of pregnant cows, but some veterinary practitioners venture to use culture 19 on all the animals in certain badly infected herds, regardless of the condition of pregnancy. At least one of the commercial firms manufacturing the vaccine has advocated such practice in its advertising literature.

In order to test the effects of strain 19 on pregnant cattle, a limited number of trials under the controlled conditions were made. As shown in table 2, three of the five cows vaccinated while pregnant, and not exposed, calved normally; a fourth cow gave birth to an apparently normal calf which died from exposure before being found; the fifth produced a weak calf which was killed and was the only one of the five to yield *Br. abortus*, strain 19, although at the time of each calving guinea pigs were inoculated with uterine material and colostrum.

Three other cows were vaccinated while pregnant approximately 4 months, and exposed conjunctivally with virulent *Br. abortus* U. C. V. 36 a month later. Table 2 shows that only one of these cows calved normally, with negative laboratory findings. The other two cows gave birth to premature calves which soon died, and *Br. abortus* was isolated from the placenta of each cow, the organism in one case showing characteristics of strain 19.

In certain field trials conducted by veterinary practitioners in cooperation with the writers, the vaccine has been used on cattle in various stages of pregnancy. The results so far have been variable. In one herd a rather high percentage of the cows aborted; the herd, however, was badly infected at the time of the vaccinations, and it has not been possible to determine if the abortions were due to natural infection or to injury from the vaccine.

WORK ON CATTLE WITH STRAIN U. C. V. 36

CHARACTERISTICS OF THE CULTURE USED

For conjunctival exposure trials to test the resistance of the vaccinated cattle, a strain of *Br. abortus* was selected which for 6 years has been proved repeatedly by the writers to be highly virulent for cattle. This strain, U. C. V. 36, was isolated in February 1931 in direct culture from the placenta of a cow that had aborted at a com-

mercial dairy farm. Its cultural characteristics correspond to those considered most typical of strains from bovine sources. Although transplanted at frequent intervals no tendency to dissociation has been observed in this culture. The colonies of this strain are always of the S type. It will not grow except in an atmosphere of increased carbon dioxide, and it shows the hydrogen sulphide and dye-plate characteristics of a typical bovine strain.

When 1 to 3 billion organisms of this culture are dropped upon the uninjured eye of a pregnant, susceptible cow a typical course of brucellosis follows, similar to that in a naturally infected animal in regard to blood and milk titers, abortion, and udder infection.

The subcutaneous injection of this culture into sheep during advanced pregnancy caused these animals to become shedders very soon after parturition. In some cases they continued for months to eliminate enormous numbers of organisms in their milk. In 1932 the culture was tried on three pregnant sows by intravenous inoculation of 200 million organisms and all aborted fetuses were infected with U. C. V. 36 type of organism. In three other pregnant sows, exposed orally or conjunctivally, the only evidence of infection was a rise in agglutination titer.

The culture has been used to infect 74 cattle by way of the conjunctivae, and the results are apparently satisfactory as a means of measuring the resistance of cattle to infection.

ANIMALS AND METHODS

To determine the immunity produced by vaccination, 19 vaccinated and 24 control cows have been exposed conjunctivally to virulent *Br. abortus* strain U. C. V. 36. At the time of exposure they were in various stages of pregnancy, ranging from 90 to 171 days. The results of these exposure trials are shown in table 3, which also contains a record of the reexposure, at a later pregnancy, of two of the vaccinated animals, and of two others exposed only by association with aborting cows. The animals are listed in four groups according to the respective dates of their exposure.

The suspension of strain U. C. V. 36 used for group 1 was prepared from slants made from beef-infusion agar containing 2 percent of glycerin and 1 percent of glucose. This medium was heavily seeded and incubated for 72 hours in the usual atmosphere of carbon dioxide. The surface growth was removed and diluted with physiological sodium chloride solution to a reading of 1 cm on a Gates opacimeter (McFarland tube 7). Each animal received a total of about 0.4 cc. The eyelids were then closed and gently massaged with the palm of the hand. The dose placed on the conjunctivae was estimated by plate counts to contain about 1½ billion living organisms.

The suspension of strain U. C. V. 36 used for group 2 was prepared in the same manner as that for group 1, with the exception that the medium was liver-infusion agar. The method of exposure consisted in placing 0.25 cc upon the sclera of each eyeball and a like amount under the lower lid of each eye. The lids were then closed and massaged vigorously for about 5 seconds. The total dosage given group 2 contained approximately 10 billion organisms, or about seven times the number used in exposing groups 1 and 4. Group 3 was not exposed conjunctivally, but by association with aborting animals. In exposing group 4, an effort was made to follow as closely as possible the dosage and technique of application used for group 1.

TABLE 3.—*Results of exposing vaccinated and control cattle to virulent Brucella abortus strain U. C. V. 36*

Group and how exposed to *Br. abortus*	Cow no.	Age when vaccinated	Period elapsed till exposed	Conditions at time of exposure: Total gestations completed	Conditions at time of exposure: Age	Conditions at time of exposure: Time pregnant	Blood agglutination titers[1]: At vaccination	Blood agglutination titers[1]: Highest caused	Blood agglutination titers[1]: When exposed	Blood agglutination titers[1]: At first positive hemoculture	Blood agglutination titers[1]: Highest after exposure	Results of hemocultures on indicated number of days after exposure[2]: 1	4	7	12	21	28	35	42	Parturition data: Type of parturition	Gestation	Time after exposure	Cultures for *Br. abortus*: Uterus	Cultures for *Br. abortus*: Milk
		Mos.	*Mos.*	*No.*	*Yrs.*	*Dys.*															*Dys.*	*Dys.*		
, conjunctival exposure on Oct. 24, 1934, to 1½ billion *Br. abortus.*	1	19	24	1	3½	113	0	1,600	100	-------	400	−	−	c	−	−	−	−	−	Vigorous calf	279	166	−	−
	2	18	24	0	3½	117	0	1,600	50	-------	200	−	−	−	−	−	−	−	−	do	276	159	−	−
	3	27	24	2	4	110	0	800	−100	-------	400	−	−	−	c	−	−	−	−	do	284	174	−	−
	4	27	24	2	4	90	0	6,400	−200	-------	400	−	−	−	c	−	−	−	−	do	281	191	−	−
	5	(3)	-------	2	5	115	-------	-------	0	-------	0	−	−	−	−	−	−	−	−	do	290	175	−	−
	6	(3)	-------	1	3	125	-------	-------	0	-------	800	−	−	−	c	−	−	−	−	Dead fetus	244	119	+	+
	7	(3)	-------	1	4	112	-------	-------	0	-------	800	−	c	c	−	−	−	c	−	do	202	90	+	−
	8	(3)	-------	1	3	92	-------	-------	0	−100	800	−	−	c	+	−	−	c	−	do	147	55	+	+
	9	(3)	-------	0	3	110	-------	-------	0	−200	1,600	−	−	c	+	−	−	−	−	do	201	91	+	+
	10	(3)	-------	0	4	114	-------	-------	50	50	3,200	−	−	−	+	+	−	−	−	do	219	105	+	+
												3	7	10	14	17	22	36	43					
, conjunctival exposure on Oct. 29, 1935, to 10 billion *Br. abortus.*	11	4	16	0	1⅔	139	0	1,600	0	-------	3,200	−	−	−	−	c	c	−	−	Dead fetus	207	68	+	+
	12	4	12	0	1¼	169	0	400	0	-------	−25	−	−	−	−	−	−	−	−	Vigorous calf	277	108	−	+
	13	19	36	1	4½	147	0	1,600	−50	−200	3,200	c	+	−	−	−	−	−	−	Dead fetus	209	62	+	+
	14	15	36	0	4¼	125	0	3,200	−100	-------	800	c	−	−	−	−	−	−	−	Weak calf	252	127	+	+
	15	15	36	1	4¼	136	0	400	0	-------	1,600	−	−	−	−	−	−	−	−	Dead fetus	222	86	+	+
	16	21	36	1	4¾	148	0	1,600	−25	-------	800	−	−	−	−	−	−	−	−	Dead calf	283	135	−	+
	17	24	12	0	3	162	0	3,200	−50	-------	1,600	−	−	−	−	−	−	−	−	Dead fetus	249	87	+	+
	18	15	36	1	4¼	-------	0	3,200	−25	-------	800	−	−	−	−	−	−	−	−	Not pregnant	-------	-------	-------	-------
	3	27	36	3	5	155	(4)	-------	50	-------	200	−	−	−	−	−	−	−	−	Vigorous calf	279	124	−	−
	4	27	36	3	5	142	(4)	-------	−200	-------	200	−	−	−	−	−	−	−	−	do	285	143	−	−
	5	(3)	-------	3	6	139	(4)	-------	0	-------	100	−	−	−	−	−	−	−	−	do	279	140	+	−
	19	(3)	-------	9	12	147	(4)	-------	−25	-------	50	−	−	−	−	−	−	−	−	do	285	138	−	−
	20	(3)	-------	1	4	157	-------	-------	0	25	12,800	−	+	+	c	−	−	−	−	Dead fetus	210	53	+	+
	21	(3)	-------	1	4	130	-------	-------	0	-------	800	−	−	−	−	−	−	−	−	Dying calf	237	107	+	+
	22	(3)	-------	0	2	164	-------	-------	0	−25	3,200	−	+	−	−	c	−	−	−	Dead fetus	209	45	+	+
	23	(3)	-------	0	2	152	-------	-------	0	-------	3,200	−	−	−	−	−	−	−	−	do	217	65	+	+
	24	(3)	-------	1	4½	169	-------	-------	200	200	6,400	−	+	+	+	−	−	−	−	do	226	57	+	+

See footnotes at end of table.

TABLE 3.—*Results of exposing vaccinated and control cattle to virulent Brucella abortus strain U. C. V. 36*—Continued

Group and how exposed to *Br. abortus*	Cow no.	Age when vaccinated	Period elapsed till exposed	Conditions at time of exposure: Total gestations completed	Conditions at time of exposure: Age	Conditions at time of exposure: Time pregnant	Blood agglutination titers: At vaccination	Blood agglutination titers: Highest caused	Blood agglutination titers: When exposed	Blood agglutination titers: At first positive hemoculture	Blood agglutination titers: Highest after exposure	Results of hemocultures on indicated number of days after exposure: 3	7	10	14	17	22	36	43	Parturition data: Type of parturition	Gestation	Time after exposure	Cultures for *Br. abortus*: Uterus	Cultures for *Br. abortus*: Milk
		Mos.	*Mos.*	*No.*	*Yrs.*	*Dys.*															*Dys.*	*Dys.*		
2. conjunctival exposure on Oct. 29, 1935, to 10 billion *Br. abortus*.—Continued	25	(3)		1	4½	171			0	−25	3,200	−	+	+	−	−	+	−	−	Dying calf	217	46	+	+
	26	(3)		1	4	166			0		800	−	−	−	−	−	−	−	−	Dead fetus	231	65	+	+
	27	(3)		4	9	165			0	25	1,600	−	−	+	−	−	−	−	−	do	223	58	+	+
	28	(3)		2	8	144			−100	−100	3,200	−	+	+	+	+	+	+	−	do	209	65	+	+
	29	(3)		1	4	152			0		1,600	−	−	−	−	−	−	−	−	Weak calf	248	96	+	+
	30	(3)		1	3½	153			0		400	−	−	−	−	−	−	−	−	do	266	113	+	−
												5	14	21	28	46	62	91						
3. in contact with aborting cows beginning Dec. 13, 1935.	31	7	17	0	2	139	0	800	0		−50	c	c	−	−	−	−	−	−	Dead fetus	186	47	−	−
	32	38	13	2	4½	131	0	1,600	−25		−100	−	−	−	−	−	−	−	−	Vigorous calf	276	145	−	−
	33	(3)		2	4½	134			0		3,200	−	−	c	−	−	−	−	−	Dead fetus	233	99	+	+
	34	(3)		0	1½				0		−100	−	−	−	−	c	−	−	−	Not pregnant				
												6	8	11	13	15	19	26	33					
4. conjunctival exposure on Nov. 6, 1936, to 1½ billion *Br. abortus*.	35	106	24	3	11	154	−100	3,200	−100		−200	−	−	−	−	−	−	−	−	Vigorous calf	286	132	−	−
	36	8	28	1	3	104	0	1,600	0		25	−	−	−	−	−	−	−	−	do	281	177	−	−
	37	39	24	2	5¼	164	−25	3,200	−200		−400	−	−	−	−	−	−	−	−	do	287	123	−	−
	38	38	24	3	5¼	117	0	3,200	−50		50	−	−	−	−	−	−	−	−	do	283	166	−	−
	39	16	28	0	3⅔	157	0	6,400	−25		6,400	−	−	−	−	−	−	−	−	Dead fetus	221	64	+	+
	40	16	28	0	3⅔	159	0	6,400	−25		3,200	−	−	−	−	−	−	−	−	do	238	79	+	+
	41	8	18	0	2⅙	115	−25	3,200	−50	−100	3,200	−	+	−	−	−	−	−	−	do	261	146	+	+
	42	(3)		0	2⅔	138			0		3,200	−	−	−	−	−	−	−	−	do	203	65	+	+
	43	(3)		0	2⅔	169			0	0	−800	+	+	+	+	−	−	−	−	do	201	32	+	+
	44	(3)		0	2½	119			0	−100	6,400	−	−	+	−	+	+	−	−	do	207	88	+	+
	45	(3)		0	2⅓	110			0	−100	6,400	−	−	+	−	+	−	−	−	do	192	82	+	+
	46	(3)		0	2	115			0	0	12,800	−	+	+	+	−	−	+	−	do	188	73	+	+
	47	(3)		0	2	167			0	0	6,400	−	+	+	−	−	−	−	−	do	220	53	+	+
	48	(3)		0	2½	163			0	−100	3,200	−	−	−	+	−	−	+	+	do	230	67	+	+

[1] Any agglutination titers shown for control animals at time of exposure were due to injections of heat-killed *Br. abortus* (bacterin) at intervals between March 1932 and June 1933.
[2] +=positive; −=negative; c=contaminated. [3] Control. [4] Second exposure. [5] Data regarding reexposed cow 19 are given on p. 125.

DISCUSSION OF TABLE 3

RESULTS WITH GROUP 1

The data for group 1 in table 3 show a marked difference in the effect of conjunctival exposure of the vaccinated cows, as compared to the controls. The only effect from exposure apparent in the vaccinated animals was a rise in the agglutination titer. It will be noted that they all gave birth to vigorous calves at full term, and the cultures of the blood, uterine material, and milk were negative. On the other hand, all but one of the controls aborted, and they also showed other effects typical of brucellosis, such as necrosis of the placenta and the presence of *Br. abortus* in the uterine material. Four controls in group 1 were proved to shed this organism in the milk and three yielded cultures of it from the circulating blood. It will be noted that control cow 5 escaped infection. During her next gestation period she was again subjected to conjunctival exposure (see group 2, table 3) and again gave birth to a full-term calf. Following her second exposure, her placenta was normal in appearance, but *Br. abortus* was isolated from it and a few weeks later her agglutination titer rose to 100. However, after a few months it subsided to negative. It is believed that she had some natural resistance to *Brucella*.

RESULTS WITH GROUP 2

There was not such a clear-cut difference between the vaccinated and the control animals in group 2, in respect to their resistance, as in group 1. This was possibly due to the fact that the cows in group 2 were exposed to about seven times the number of organisms used on those in group 1 and also to the more vigorous massaging of the eyelids in group 2. Five of the vaccinated animals developed brucellosis and the organism was isolated in each case from the placenta and milk. Of the three which failed to show evidence of the brucellosis, one was nonpregnant, one gave birth to a dead calf at full term, and only one (no. 12) produced a vigorous calf. The 11 controls (nos. 20–30), exposed for the first time, developed typical brucellosis.

In group 2 were four animals which had been previously exposed. The record of three of these (nos. 3, 4, and 5) is evident from the table, as they also appear in group 1. The fourth (no. 19) had been fed *Br. abortus* 9 years previously, when she was pregnant 114 days, which resulted in an abortion, and at that time *Br. abortus* was isolated from her placenta and milk. Thereafter her agglutination titer remained positive for 2 years, and suspicious or negative for another 2 years. The resistance shown by the four animals subjected to reexposure is so definite, in comparison with the infection of the others in this group, that one can conclude that the infection with the virulent *Br. abortus* eventually produced a much stronger resistance than did the subcutaneous vaccination with strain 19.

RESULTS OF CONTACT EXPOSURE IN GROUP 3

When the animals in this group were supposed to be 131 to 139 days pregnant, they were tested for immunity by placing them in a corral in which animals from group 2 were aborting.

One of the vaccinated cows (no. 32) gave birth to a normal, vigorous calf, and laboratory examinations for *Br. abortus* were negative.

The other (no. 31) aborted a dead fetus on the one hundred and eighty-sixth day of gestation, but, as cultures and guinea pig inoculations of uterine material, fetal tissue, and colostrum, as well as milk, were all negative for the organism, the abortion is believed to have been caused by some factor other than infection contracted through exposure to aborting cows.

One of the control animals developed an agglutination titer of —100 following contact with aborting cows, but was not found to be pregnant. The other control cow aborted a dead fetus on the two hundred and thirty-third day of gestation, and *Br. abortus* was recovered from her uterine, fetal, and udder material. The agglutination titer of this cow, which had previously been negative, rose to 1,600 at the time of abortion, in marked contrast to the low titers developed by the vaccinated cows subjected to exposure in this group.

RESULTS WITH GROUP 4

In table 3 it may be seen that group 4 consisted of seven vaccinated and an equal number of control cows. It is noteworthy that the vaccinated animals in this group that calved normally had previously borne calves, while the other three that aborted, as well as all of the controls, were primiparous.

The fact that only one of the vaccinated animals in this group yielded a positive blood culture, while six of the controls repeatedly yielded positive cultures, confirms the hemoculture results previously obtained from the animals in groups 1 and 2 and would justify the conclusion that vaccination tends to reduce the bacteraemia following exposure to *Br. abortus*.

All group 4 animals were kept continuously at pasture from the date of their weaning to the time they calved or aborted in the fall of 1936 or the early months of 1937. In this respect their treatment was similar to that given groups 1 and 2. The weather conditions during 1936, however, were more unfavorable to the nutritive qualities of the pasture vegetation than in the previous 4 years. Little rain fell between June and December 1936, and the pasture grasses (wild oats, alfilaria and bur clover) became unusually dry and bleached. Furthermore, the first fall rains were followed by such cool weather that no green forage developed until after the first of January. From November 1936 to February 1937 sparing amounts of alfalfa hay were fed two or three times a week to the animals in this group. This supplementary feeding provided a better diet than that available to the average heifer or nonlactating cow in the coast ranges of California, and at the time of exposure of group 4 the diet was considered to be adequate. It was not adequate, however, for tests by the antimony trichloride method on liver tissue from some of the fetuses showed complete lack of color reaction for vitamin A, whereas the livers of certain calves born to cows not in the experiment but kept in the vicinity and fed concentrates and all the alfalfa hay they would eat, showed a relatively high content of vitamin A.

It is probable that a combination of the effects of *Br. abortus* infection and a low intake of vitamin A, and possibly other dietary essentials, were responsible for the abortions in group 4. The results are significant as an indication of what might occur in vaccinated cattle exposed to infection under average conditions of winter range in the San Francisco Bay section.

Hart and Guilbert (*9*) have demonstrated that a lack of vitamin A in range grasses in California may cause a condition in cows resulting in the birth of dead or weak calves and retained placentas, a condition simulating infectious abortion, although the cattle may be free from brucellosis. That the pathogenic effects of *Brucella* infection may be enhanced by a lack of vitamin A in the diets of cattle is indicated by the results in group 4.

SUMMARY

Since October 1932 cultures of *Brucella abortus*, United States Bureau of Animal Industry strain 19, have been under observation. When grown on glucose-glycerin agar, the strain has apparently remained unchanged in its relatively low virulence for guinea pigs and cattle.

When grown in plain broth for about 4 weeks the strain became dissociated so that transplants to glucose agar resulted in a predominance of R-type colonies, but, when occasionally renewed by planting from S-type colonies, it remained sufficiently stable to be satisfactory for the production of antigen for the detection of agglutinins by the tube and the plate methods.

After subcutaneous injection into cattle in doses of approximately 200 billion organisms, the strain has been isolated from the circulating blood up to the twenty-second day, and from the subcutaneous tissues near the point of injection at 14 and 24 days. When injected subcutaneously into pregnant cows, it may produce typical brucellosis with transient bacteraemia, exudative and necrotic placentitis, with the discharge of large numbers of the organisms in uterine material at the time of parturition.

Cattle vaccinated with strain 19 while nonpregnant have been kept closely associated with nonvaccinated cattle for over 3 years without any evidence of the transmission of infection to the nonvaccinated animals.

The vaccination of mature nonpregnant heifers produced, for at least 2 years, a very definite resistance against the effects of exposure from introducing 1½ billion virulent *Br. abortus* into the conjunctival sacs. This resistance may be overwhelmed when larger doses are introduced into the conjunctival sacs, followed by vigorous massage of the eyelids.

Infection of adult cows with virulent strains of *Br. abortus* produced a stronger resistance to subsequent infection than was produced in younger cattle by vaccination with strain 19.

The data on results of vaccination between the ages of 4 and 16 months are too meager to permit definite interpretation, but they tend to indicate that the agglutination titer usually vanishes within a year and that only a small percentage of such animals are sufficiently protected to withstand severe conjunctival exposure when they are maintained on the low vitamin A intake available in the fall and winter months on the average California coast range.

The results from hemocultures indicate that calfhood vaccination with strain 19 tends to inhibit the bacteraemia that frequently follows conjunctival exposure of cattle with virulent *Br. abortus*.

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FURTHER STUDIES ON THE INTERRELATIONSHIP OF INSECTS AND FUNGI IN THE DETERIORATION OF FELLED NORWAY PINE LOGS[1]

By J. G. LEACH, *associate plant pathologist*, L. W. ORR, formerly *instructor in forest entomology*, and CLYDE CHRISTENSEN, *assistant forest pathologist, Minnesota Agricultural Experiment Station*[2]

INTRODUCTION

In an earlier paper[3] the authors outlined plans for a study of the interrelationships of insects and fungi in the deterioration of felled timber and described the association of two species of bark beetles with two species of blue-staining fungi. In this paper are recorded observations and experiments on several species of wood-boring beetles and their influence on the development of heart rot in felled Norway pine logs.

METHODS AND PROCEDURE

It will be necessary here only to describe briefly the general plan of the experiment. For further details the reader is referred to the earlier paper.[3] On May 18 and 19, 1931, 56 logs of Norway pine (*Pinus resinosa* Ait.), 7 to 12 inches in diameter and 40 inches in length, were cut. They were divided into 5 series of 8 logs each and a check series of 16 logs and were given the following treatments:

A. No treatment; not caged (check series).
B. Ends and limb scars disinfected with a 2-percent aqueous solution of ethyl mercury chloride, then sealed with roofing pitch and covered with burlap; not caged.
C. Same as series B, but enclosed in a cage of 18-mesh aluminum-coated screen wire.
D. No end treatment, but enclosed in a cage of 18-mesh screen wire.
E. Ends and limb scars sprayed at frequent intervals during the first two summer seasons with a 2-percent aqueous solution of ethyl mercury chloride; not caged.
F. Same as series E, but enclosed in a cage of 18-mesh screen wire.

After treatment the logs were placed in position on supports a few inches above the ground under a slanting roof of laths providing approximately 50 percent shade. The end treatments were intended to prevent fungus infection by wind-borne spores and the cages were to exclude insects and any fungi that they might introduce. The nontreated and noncaged logs of the check series were exposed to both insect attack and wind-blown fungus spores. By observing the relative amounts of decay and insect infestation in the logs of the different series it was hoped that some indication of the influence of insect infestation on the development of decay could be obtained.

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[2] The authors express their appreciation to Dr. Alexander C. Hodson for his assistance in checking the identity of the insect species mentioned in this paper and for critically reading the manuscript.

[3] LEACH, J. G., ORR, L. W., and CHRISTENSEN, C. THE INTERRELATIONSHIPS OF BARK BEETLES AND BLUE-STAINING FUNGI IN FELLED NORWAY PINE TIMBER. Jour. Agr. Research 49: 315-342, illus. 1934.

In addition to the above experiment, other logs were cut, caged, and treated as in series C and F. Into these cages varying numbers of different species of insects were introduced. In this way the development of individual species of insects and associated fungi was studied without being complicated by the presence of other insects or fungi. These served also as material for studying the various stages in the life cycle of the insects.

GENERAL OBSERVATIONS ON INSECT ATTACKS AND FUNGUS INFECTIONS

Bark beetles were the first insects to attack the experimental logs. The development of two species of these beetles and their association with certain blue-stain fungi have been described.[3] Shortly after the bark beetles had entered the logs, several species of wood-boring insects made their appearance. The most prevalent of these were the cerambycid beetles *Monochamus scutellatus* (Say), and *M. notatus* (Drury), and two buprestids, *Chalcophora virginiensis* (Drury), and *Chrysobothris dentipes* (Germar). The activities of these species and their relation to the development of wood rot in the logs are described in this paper.

In addition to these, a few other species were found in the logs in varying numbers. Since most of the latter group confined their activities to the bark and outer layers of the sapwood and made their appearance after the logs were thoroughly infested with bark beetles, no direct association with any specific fungus could be observed. A few experiments were made with some of these insects in caged and sealed logs, but no clear-cut associations with fungi were established. The most prevalent of these insects were the following:

1. *Rhagium lineatum* Oliver. This was common in most of the noncaged logs. Development was confined to the inner bark and outer sapwood. Numerous and varied species of fungi were isolated from the frass and tunnels, but no one species was consistently present, and since all the surrounding tissues had been thoroughly infested by secondary fungi, no definite associations could be detected.

2. *Hylobius congener* D. T., S., and M., a weevil, was found in several of the exposed logs. The weevils were usually near the ends of the logs and made their tunnels in the inner bark and the surface of the sapwood. Their activity was very limited and there was no consistent decay or discoloration of the sapwood associated with them. Although the frass found in the pupal chambers was frequently contaminated with numerous molds, these usually could be detected only by culturing. It is to be concluded that these insects have little or no effect on the development of fungi in the logs.

THE RELATION OF MONOCHAMUS SCUTELLATUS AND M. NOTATUS TO THE DECAY OF HEARTWOOD

Monochamus scutellatus and *M. notatus* were the most prevalent wood-boring insects found in the logs. From 2 to 15 larvae of these species were found in nearly every log that was not caged. Both species were present, *M. scutellatus* being the most abundant, but it was not always possible to distinguish the species in the larval stage. Their life histories and activities in the logs were essentially similar. For these reasons they are considered together in the following discussion. Observations and experimental data showed that the presence of these insects in the logs markedly influenced the rate of decay of the heartwood.

[3] LEACH, J. G., ORR, L. W., and CHRISTENSEN, C. THE INTERRELATIONSHIPS OF BARK BEETLES AND BLUE-STAINING FUNGI IN FELLED NORWAY PINE TIMBER. Jour. Agr. Research 49: 315-342, illus. 1934.

These species of *Monochamus* infest freshly felled pine logs or pine trees that have been killed or severely injured by some other agency. The adults are elongate, somewhat cylindrical, black or brown beetles about 1 inch in length. Individuals of each species differ considerably in size and color. The females are somewhat lighter in color than the males and are marked with irregular grayish-white spots (fig. 1, *B*).

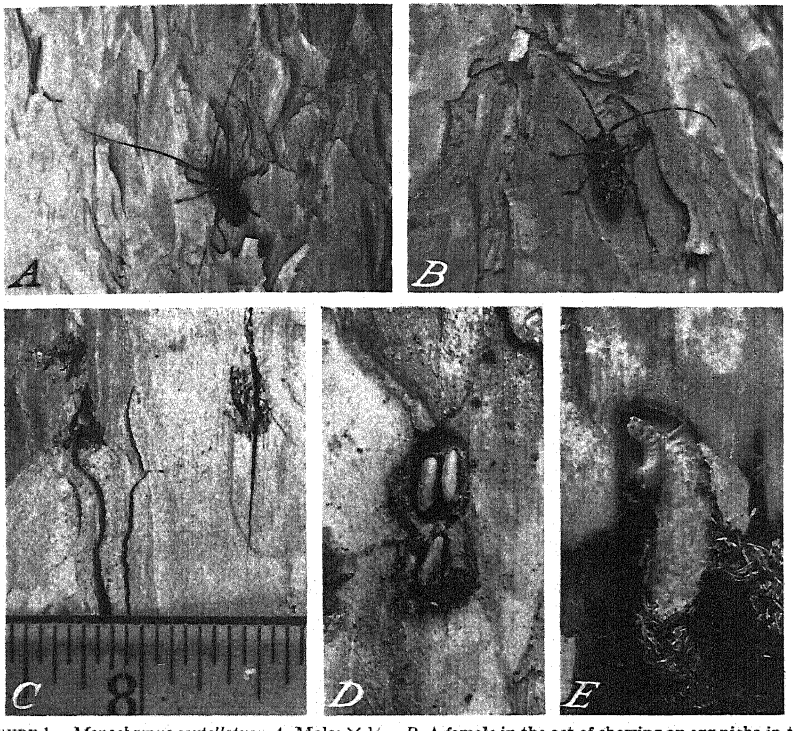


FIGURE 1.—*Monochamus scutellatus: A*, Male; × ½. *B*, A female in the act of chewing an egg niche in the bark of a Norway pine log; × ½. *C*, Three egg niches; × 1¼. *D*, Three *Monochamus* eggs exposed by removing the bark around a niche; × 2½. *E*, A young *Monochamus* larva feeding in the bark of a Norway pine log; × 1½.

The antennae of the males are somewhat longer than those of the females. The adults emerge from infested logs in spring and early summer. During a flight period of several weeks they feed upon the leaves or green bark of pine twigs. Oviposition reaches its peak shortly before midsummer. The eggs are deposited in slits that the females make in the bark with their mandibles (fig. 1, *C*). The slits extend into the living inner bark, and from one to three eggs are deposited in each slit (fig. 1, *D*). The larvae emerge from the eggs and tunnel in the inner bark and cambium region (fig. 1, *E*). If more than one egg hatches, usually only one larva survives, probably because of cannibalism. As the larva increases in size the excavation is enlarged and extends for a depth of a few millimeters into the sapwood (fig. 2, *A*). As the larva approaches maturity, it turns inward and bores an ovoid tunnel through the sapwood and into the heartwood for a considerable depth. Before pupation, the larva turns and bores toward the upper surface of the log. The pupal

chamber is formed about 1 inch below the surface. When the adult is formed it bores out its own emergence tunnel and emerges usually at or near the upper surface of the log through a round hole about one-quarter to three-eighths of an inch in diameter (fig. 2, *B*). The

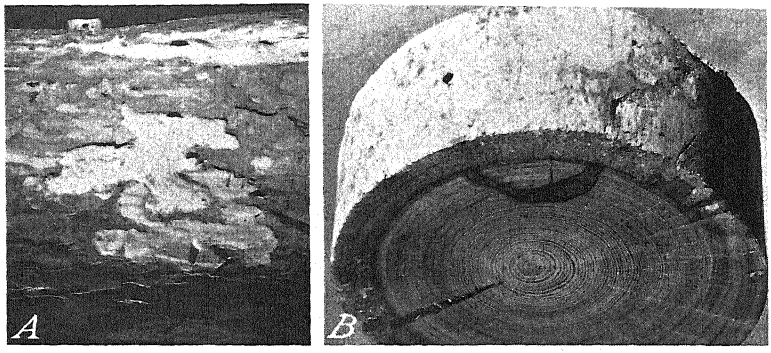


FIGURE 2.—*A*, Portion of a Norway pine log with bark removed to show feeding and penetration tunnels of two *Monochamus* larvae; *B*, section of a log (center section of log 39) showing penetration and exit tunnel of a *Monochamus* beetle. Note the decay that invaded the heartwood through the tunnel in a radial and tangential direction and spread for a considerable distance up and down the log.

time required for completion of the life cycle varies from 1 to 3 years. It is a significant characteristic of this insect that the larva tends to keep the tunnel clear of frass and chips by casting these out through holes that it makes in the bark (fig. 3, *B*).

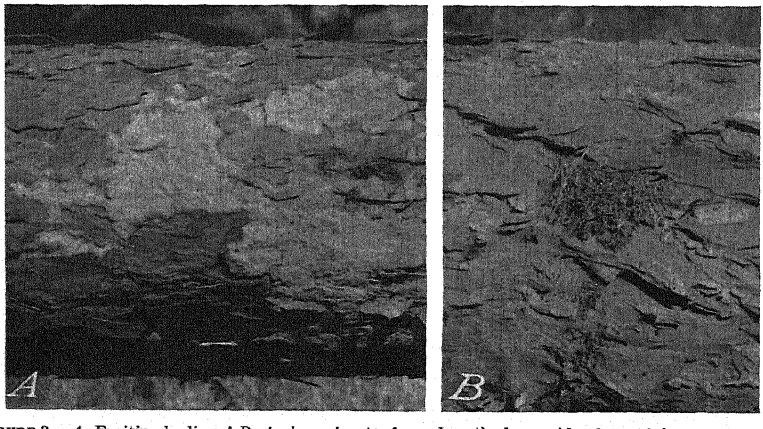


FIGURE 3.—*A*, Fruiting bodies of *Peniophora gigantea* formed on the lower side of one of the experimental logs; *B*, pile of frass pushed out of the tunnel of a *Monochamus* larva. *Monochamus* larvae keep their tunnels open by pushing all frass out through a hole made in the bark.

Graham,[4] in a study of the ecology of insects in felled logs, observed that wood rots were commonly associated with the tunnels of this insect, and suggested that the open nature of the tunnels offered ideal conditions for infection of the logs with wood rots. That there is some relation between infestations by these beetles and the decay of the heartwood is clearly demonstrated by the results of the present experi-

[4] GRAHAM, S. A. THE FELLED TREE TRUNK AS AN ECOLOGICAL UNIT. Ecology 6: 397–411, illus. 1925.

ment. In table 1 are given the summarized data on the two series of logs showing the wood-boring insects present and the corresponding amount of heartwood decay for each treatment. One of these series was examined in August 1933 and the other in September 1934, three and four summer seasons respectively after the beginning of the experiment. The insect counts were made immediately after the bark was removed from the log. The amount of heartwood decay was determined by measuring the area of decayed heartwood exposed in representative logs of each series when cut in cross or longitudinal sections, as shown in figure 4. The areas were measured with a planimeter and

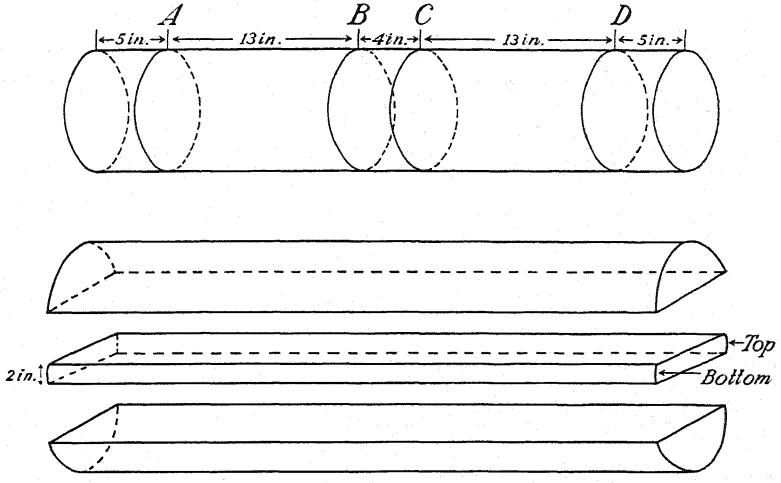


FIGURE 4.—Diagram showing how logs were cut for the measurement of the amount of heartwood decay

calculated in terms of percentage of the total area of heartwood exposed in the section. A study of the table will show that there is a fair degree of correlation between the number of wood-boring insects, especially *Monochamus* spp., and the amount of heartwood decay in the log. The correlation is shown more clearly in figures 5 and 6. It will be noted that the greatest lack of correlation is in series D and F. The logs were caged in both series. In series D the ends were given no treatment and in series F the ends were sprayed with ethyl mercury chloride at intervals during the first two summer seasons but received no treatment afterward. By the time the logs were examined the ends were badly checked, the cracks extending far into the heartwood; and it was obvious that the wood rot had spread from the sapwood deep into the heartwood through these cracks. In practically all cases in these series, as well as in series A, where the ends were exposed also, the amount of decay in the end sections was much greater than in the center sections. These differences are shown in figures 5 and 6, where the percentages of decay in the end sections are compared with those of the center sections.

The significance of the exposed ends and the deep checks that formed on the exposed ends can be more fully appreciated when they are considered in connection with the relative rate of spread of the decay in different directions in the heartwood. It was not possible to make carefully controlled measurements of the rate of spread of

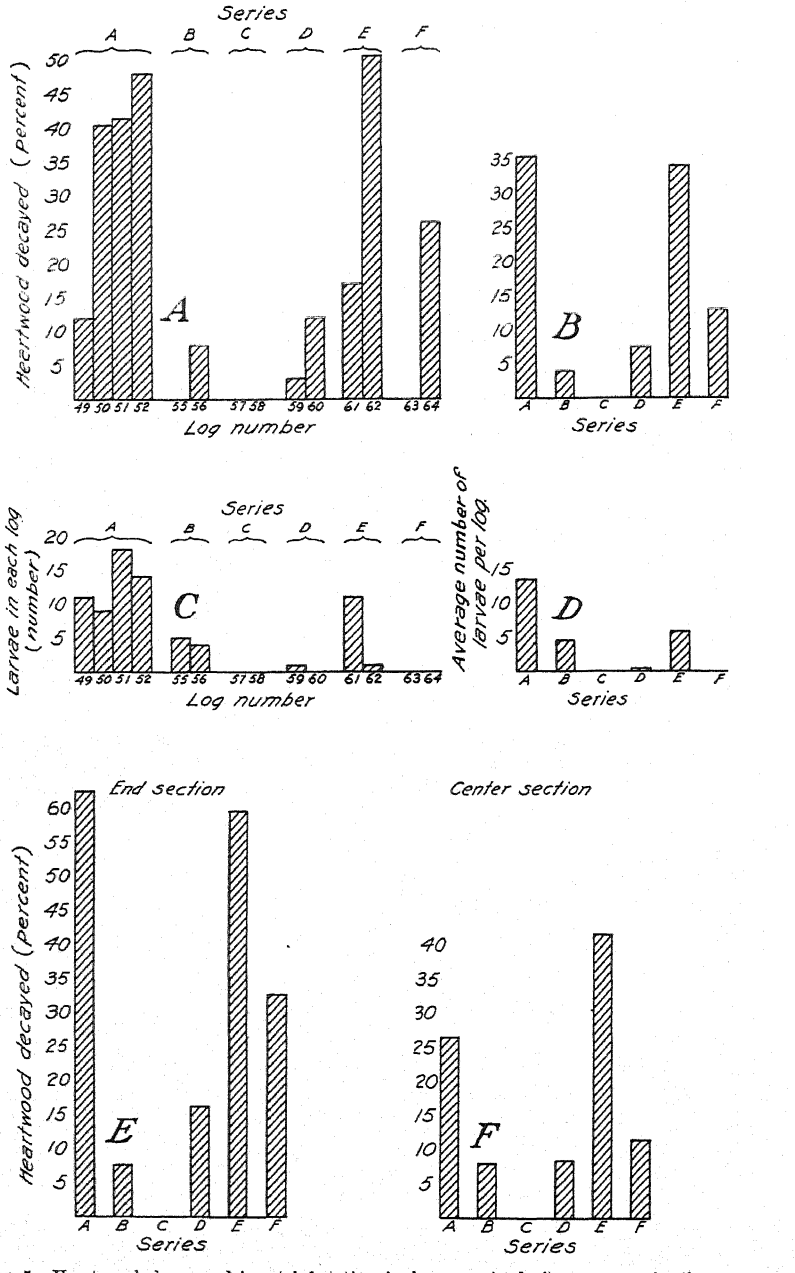


FIGURE 5.—Heartwood decay and insect infestation in logs examined after exposure for three seasons, August 1933: *A*, Total decay for each log; *B*, total decay for logs in each series; *C*, larvae of *Monochamus* spp. found in each log; *D*, larvae of *Monochamus* spp. found in logs in each series; *E*, percentage of decay in end sections of logs; *F*, percentage of decay in center sections of logs. See text for description of treatments used in each lettered series.

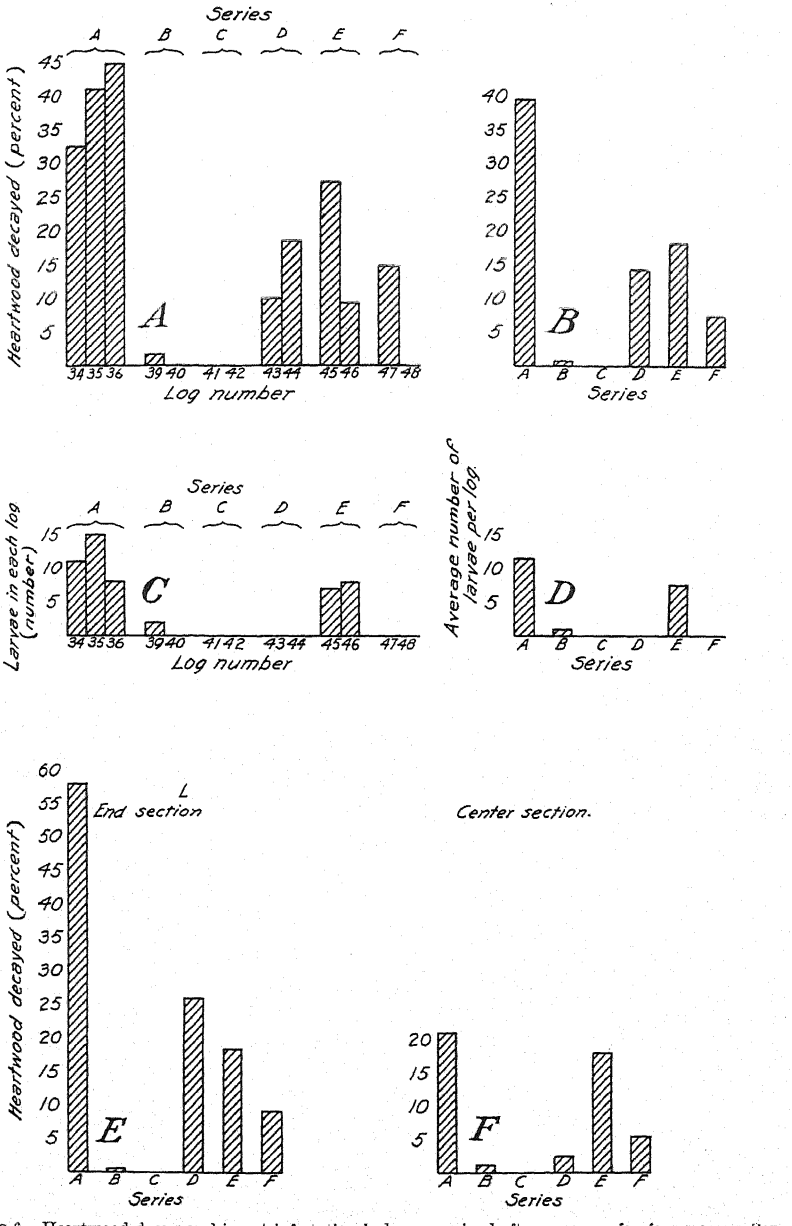


FIGURE 6.—Heartwood decay and insect infestation in logs examined after exposure for four seasons, September 1934: *A*, Total decay for each log; *B*, total decay for logs in each series; *C*, larvae of *Monochamus* spp. found in each log; *D*, larvae of *Monochamus* spp. found in logs in each series; *E*, percentage of decay in end sections of logs; *F*, percentage of decay in center sections of logs. See text for of description treatments used in each lettered series.

decay in the logs; but when the logs were cut and examined, it was perfectly obvious that the decay in the heartwood spread very much more rapidly in a longitudinal direction than either radially or tangentially. This difference was less marked in the sapwood, which was nearly always completely decayed before any appreciable decay appeared in the heartwood. Thus, in the logs with sealed ends, there was much less sapwood decay and the decay spread from the sapwood into the heartwood by radial growth very slowly except where it followed insect infestation.

TABLE 1.—*The amount of infestation by wood-boring beetles and the amount of heartwood decay in the experimental logs*

	Infestations per log																	
	Treatment A, 8 logs			Treatment B, 4 logs			Treatment C, 4 logs			Treatment D, 4 logs			Treatment E, 4 logs			Treatment F, 4 logs		
	Minimum	Maximum	Average	Minimum	Maximum	Average	Minimum	Maximum	Average	Minimum	Maximum	Average	Minimum	Maximum	Average	Minimum	Maximum	Average
	No.	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*
Monochamus spp.	7.0	18.0	11.6	0.0	5.0	2.7	0.0	0.0	0.0	0.0	0.0	0.0	1.0	11.0	6.7	0.0	0.0	0.0
Chrysobothris dentipes	.0	6.0	.7	.0	24.0	9.0	.0	2.0	.5	.0	1.0	.2	.0	4.0	1.0	.0	1.0	.2
Chalcaphora virginiensis	.0	.0	.0	.0	.0	.0	.0	.0	.0	.0	.0	.0	.0	1.0	.2	.0	.0	.0
Hylobius congener	.0	6.0	2.4	.0	.0	.0	.0	.0	.0	.0	13.0	6.2	.0	2.0	.5	.0	2.0	.5
Unknown cerambycid	.0	7.0	.9	.0	.0	.0	.0	.0	.0	.0	.0	.0	.0	10.0	3.2	.0	.0	.0
Unknown buprestid	.0	.0	.0	.0	.0	.0	.0	7.0	2.0	.0	.0	.0	.0	.0	.0	.0	.0	.0
Rhagium lineatum	.0	.0	.0	.0	.0	.0	.0	.0	.0	.0	.0	.0	.0	3.0	.7	.0	.0	.0
	Pct.	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*
Decay of heartwood	12.5	48.3	36.8	.0	7.9	2.4	0.0	0.0	0.0	3.0	18.5	11.0	9.2	50.5	26.0	0.0	22.2	9.3

It will be observed that the amount of heartwood decay was less, and the number of *Monochamus* beetles was smaller, in the logs with sealed ends than in those with exposed ends. Nevertheless, within the series comprising logs with sealed ends, there is a close correlation between the number of *Monochamus* beetles and the amount of heartwood decay. The greater amount of heartwood decay in the logs with exposed ends was obviously caused in part by the increased opportunity for infection through the ends. However, the moisture content of the sealed logs was much higher than that of the logs with exposed ends and probably made the conditions less favorable for both insect and fungus. The moisture content of the logs was not measured after the first year, but during the first year the average loss in weight of the logs with exposed ends was 10.69 percent, and that of the logs with sealed ends only 1.75 percent. A somewhat similar difference was probably maintained during the remainder of the period. The moisture content of the caged logs was slightly higher than that of the logs not caged, but it was not high enough to inhibit the development of decay. During the course of the experiment the progressive development of the insects and decay was studied in additional caged logs in which a known number of insects were introduced. In these caged logs the decay of the heartwood in association with *Monochamus* larvae developed equally as well as it did in the exposed logs used in the comparative test.

The principal fungus causing the decay of the heartwood was *Peniophora gigantea* (Fr.) Massee. Numerous isolations were made from decayed heartwood, including many adjacent to the tunnels of *Monochamus* larvae, in various stages of development, and this species was most frequently isolated. *Polyporus anceps* Peck, *Polyporus abietinus* (Dicks.) Fries, and *Merulius* sp. were sometimes isolated, but these were very rare. The fruiting bodies of *P. gigantea* were formed on the lower side of all logs that showed an appreciable amount of decay (fig. 3, *A*). This fungus has been recognized for a long time as the cause of sapwood decay of coniferous wood but has not been considered as seriously affecting heartwood. Although growing more slowly on heartwood than on sapwood, it is obvious from these experiments that it must also be considered as a factor in heartwood decay.

Although figures 5 and 6 show that there is a relationship between the amount of heartwood decay and infestation by the *Monochamus* beetles, the most striking proof of the association is obtained by examining the insect tunnels and the areas of heartwood decay as

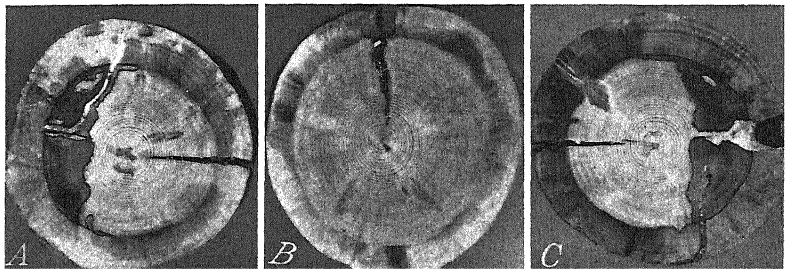


FIGURE 7.—*A*, The center section from log 45, showing two areas of decay in the heartwood that were closely associated with *Monochamus* tunnels. *B*, The center section of log 48, caged to exclude insects, showing absence of decay. In all other respects similar to log 45. *C*, The reverse side of the center section of log 45 shown in *A*.

revealed in the logs when cut in cross section. The close association of the decayed areas with the larval tunnels leaves no doubt of the fact that the decay of the heartwood is hastened by the insect infestation. This close association is well illustrated in the center sections cut from logs 39 and 45, shown in figure 2, *B*, and figure 7. The total amount of decayed heartwood resulting from each insect tunnel is not evident when seen in cross section. It is only by determining the extent of spread in a longitudinal direction that the total volume can be estimated. In log 39 (fig. 2, *B*) the decay extended more than 20 inches, and in log 45 (fig. 5) it extended the entire length of the 40-inch log.

The close association of heartwood decay with the tunnels of *Monochamus* larvae suggests the possibility that the insects may be concerned in the dissemination of *Peniophora gigantea*. If the fungus were introduced into the log by these insects it would most likely be introduced at the time of oviposition. The method of oviposition has already been described. A large number of freshly laid eggs were aseptically removed from the niches and cultured on agar. More than half of the eggs so cultured proved to be surface-contaminated with fungi. The isolations were made in 3 different years. The first year

an unidentified species of *Sporotrichum* was obtained from almost every egg. Several miscellaneous fungi were also obtained. These included *Penicillium*, *Cladosporium*, *Macrosporium*, *Fusarium*, and several kinds of yeast. In subsequent years the *Sporotrichum* was rarely found, and no other fungus was obtained with any degree of consistency. No culture resembling *Peniophora* or any other Basidiomycete was obtained.

Some of the eggs proved surface-sterile; and when the larva hatched, it also was sterile. Paraffin sections of eggs and young larvae were cut and stained, and here also no indication of internally borne fungi was found.

Cultures were made from the mouth parts and ovipositors of egg-laying females. These yielded yeasts, bacteria, and the usual assortment of molds; but no Basidiomycetes were found. Cultures made from the frass of young larvae in sound sapwood also failed to yield the wood-rotting fungus. These results led to the conclusion that these insects are of little or no importance in the dissemination of *Peniophora gigantea* or other wood-rotting fungi. *P. gigantea*, which fruits abundantly on the bark of decaying pine logs, is probably wind-disseminated, gaining access to the sapwood through holes in the bark made by insects or other means. Once under the bark, it finds the sapwood a favorable medium for growth. The sapwood is rapidly decayed, but the rate of invasion of the fungus is greatly decreased as soon as it reaches the heartwood. It can advance only very slowly in a radial or tangential direction unless aided by the larvae of *Monochamus* spp. or similar insects or by checks or cracks in the heartwood. The *Monochamus* larvae bore through the decayed sapwood and penetrate deep into the heartwood. The tunnel is a favorable place for fungus growth, and the larva, by frequent movement from one end of the tunnel to the other in pushing out its frass, is obviously a very effective aid to radial invasion by the fungus. Radial penetration having been effected, the fungus spreads rapidly through the heartwood in a longitudinal direction. It may be concluded, then, that *Monochamus scutellatus* and *M. notatus* are of little significance as agents of dissemination and inoculation of *P. gigantea*, but that in the larval stage they greatly hasten the invasion of the heartwood by the fungus.

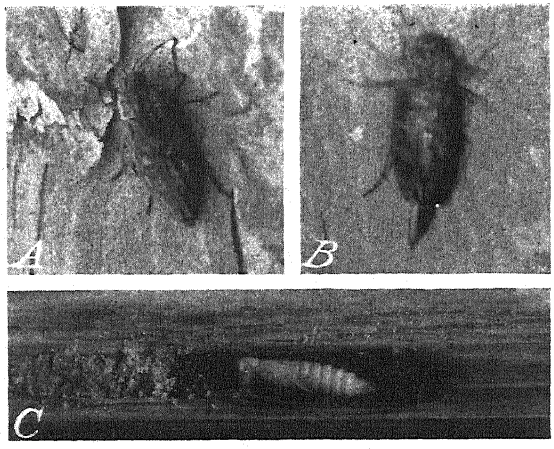


FIGURE 8.—*Chrysobothris dentipes*: *A*, Female on bark of a Norway pine log; × 1¾. *B*, The same in the act of ovipositing in a crevice in the bark; × 1¾. *C*, A longitudinal section through a tunnel showing pupa; × 1½. Note the frass tightly packed in the tunnel. The closed nature of the tunnel resulting from the nonremoval of frass probably accounts, in part, for the slight influence of this insect on the development of heartwood decay.

THE RELATION OF BUPRESTID BEETLES TO THE DECAY OF HEARTWOOD

It will be noted in table 1 that a considerable number of buprestid larvae were found in the logs. These consisted mainly of *Chrysobothris dentipes* (fig. 8), a smaller number of *Chalcophora virginiensis*, and several larvae of an unidentified species. There is very little correlation between the number of these beetles in a log and the amount of heartwood decayed. An examination of the tunnels made by these larvae also reveals no consistent association with the decayed heartwood.

The buprestid beetles lay their eggs in crevices under the outer bark. The eggs hatch and the young larvae bore into the inner bark and feed for most of their developmental period in the cambium region. Later they burrow into the sapwood and occasionally penetrate the heartwood but usually they do not go so deep as the *Monochamus* larvae. They develop more slowly and often do not pupate the first season; 2 or 3 years are required for completion of the life cycle.

The activity of these larvae differs from that of the *Monochamus* larvae, chiefly in the disposition of frass. This difference probably accounts, to a large extent, for the limited influence of the buprestid larvae on heartwood decay. The buprestid larvae, unlike the *Monochamus* larvae, do not remove the frass from their tunnels. The frass is packed in the tunnel behind the larvae as they advance. The tunnel is always closed and the larvae do not move to and fro in it (fig. 8, *C*). Cultures made from the frass taken from buprestid tunnels yielded a variety of yeasts and molds. No *Peniophora* or other Basidiomycete was isolated from buprestid tunnels, while *Peniophora gigantea* was abundant in the tunnels of *Monochamus* larvae.

The failure of the wood-rotting fungi to develop in the buprestid tunnels may be due to several factors. The moisture content of the frass filling the tunnels is usually very high and may be too high for good growth of the wood-rotting fungi. The wood adjacent to the tunnels also is usually rather moist. Tunnels filled with closely packed frass obviously offer less opportunity for loss of water from the adjacent wood. It is also possible that the yeasts and molds found in the frass or possibly some excretory product of the larva inhibits the development of the wood-rotting fungi.

DISCUSSION

When a Norway pine tree is felled, it immediately becomes susceptible to insect invasion. The first insects to attack the felled logs are bark beetles. As previously shown,[5] these insects introduce yeasts and certain blue-staining fungi. The life cycle of the bark beetles is short and they are found in the log only one season. Certain wood-boring beetles also attack the log soon after it is felled. The life cycle of the wood-boring beetles is longer, and these insects may be found in the logs for 2 or 3 years. During this period practically all of the sapwood and much of the heartwood of infested logs decay. No evidence has been obtained in this study to indicate that the wood-destroying fungi are introduced by the wood-boring beetles, but

[5] LEACH, J. G., ORR, L. W., and CHRISTENSEN, C. See footnote 3.

the two species of *Monochamus* do hasten the decay of the heartwood by providing a means for more rapid advance of the fungus or fungi through the wood in radial and tangential directions. The fungi, without such aid, spread very slowly in the heartwood radially and tangentially but more rapidly longitudinally. Thus the insect infestation, by hastening radial and tangential invasion, greatly increases the rate of heartwood decay. Here we have an unusual insect-plant disease relationship. The insect has no part in the usual role of dissemination or inoculation but is actively concerned with the process of invasion.

The other species of insects observed in the logs generally confined their activities to the inner bark and outer layers of the sapwood. Usually they do not appear until this region is thoroughly infested by miscellaneous fungi; they apparently have very little influence on the decay of the log.

SUMMARY

As a part of a study of the interrelation of insects and fungi in the deterioration of Norway pine logs, the changes occurring in felled logs during the second and third years after felling were studied. During this period, in fully exposed logs, the entire sapwood and a considerable portion of the heartwood were decayed. *Peniophora gigantea* was primarily responsible for the decay of both sapwood and heartwood.

No evidence was obtained to show that this fungus was dependent upon insects for dissemination or ingression. It is apparently disseminated readily by wind and may enter through cracks in the bark or through holes made by several species of insects.

The decay develops very rapidly in the sapwood but advances more slowly in the heartwood. Its spread in the heartwood is especially slow radially and tangentially but is more rapid in a longitudinal direction.

Several different species of insects were found in the logs during the period. Two species of cerambycid beetles (*Monochamus scutellatus* Say and *M. notatus* Drury) and two species of buprestids (*Chrysobothris dentipes* Germar and *Chalcophora virginiensis* Drury), were the most prevalent wood-boring beetles.

There was a fair degree of correlation between the number of *Monochamus* beetles in the logs and the amount of heartwood decayed. The larvae of these insects appear to hasten the decay by facilitating the radial and tangential invasion of the heartwood by *Peniophora gigantea*. The open larval tunnels formed by these insects are especially favorable for the spread of the decay.

The buprestid beetles have little influence on the rate of decay of the heartwood. The closed buprestid tunnels appear to be less suited for the spread of decay, and possibly this condition is largely responsible for the failure of these insects to influence the rate of decay.

Other species that inhabit the bark and outer layers of the sapwood appear to have little influence on wood decay.

STUDIES ON THE INHERITANCE AND DEVELOPMENT OF FRUIT SIZE AND SHAPE IN THE TOMATO[1]

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INTRODUCTION

The investigation described herein is concerned with the inheritance and the development of fruit size and shape in tomatoes. Locule number of the fruit, because of its apparent association with size and shape, was given special attention.

HISTORICAL REVIEW

The tomato was one of the early plants used in genetical studies after the rediscovery of Mendel's law, and shape was among the first characters mentioned. Hedrick and Booth (*4*)[3] as early as 1907 listed a shape factor. Price and Drinkard (*14*) mention round as dominant to pear shape, round-conic dominant to round-compressed, two-celled fruit dominant to many-celled, and smooth surface dominant to rough.

Various hypotheses have been advanced as to the number and relation of genes to size and shape. Frimmel (*2*) held that fruit size is complex, depending on carpel size and the degree of fasciation, with carpel size intermediate in F_1, and fasciation recessive. Lesley and Rosa (*7*) stated that oblateness is inevitable for size. Lindstrom (*8*, *11*) reported genes *Pp* and *Yy* linked with a major size factor. Later (*9*, *10*) he suggested as allelomorphs oval, round, and oblate linked with *Dd* and *Pp*, and reported shape and size correlated. MacArthur (*13*) gave 10 linkage groups involving 20 genes, but did not include locule number as one of these. Currence (*1*) reported significant association between fasciation and fruit size, suggesting that factors affecting fruit contour probably also affect size.

Sinnott (*15*), working with *Cucurbita*, traced the histological development of shape and size. He found shape determined in the earliest primordia of the pistillate flower, and that cell division had ceased at the time of anthesis. Houghtaling's (*5*) studies with tomato have supplied the reason for the correlations between ovary and fruit measurements reported by Hackbarth et al. (*3*) in crosses between *Lysopersicon Mill. esculentum* and *L. racemigerum*. She found that a differential rate of growth occurs at earliest primordia, ceasing in *L. esculentum* at anthesis, at which time cell division is complete.

There has been some confusion in the use of terms. The fasciation reported by Frimmel (*2*), Warren (*18*), and Houghtaling (*5*) evidently

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[2] Acknowledgment: In the pursuance of this investigation and the preparation of this manuscript, the invaluable advice and assistance of Dr. E. W. Lindstrom have been freely given and are gratefully acknowledged.

[3] Reference is made by number (italic) to Literature Cited, p. 152.

refers to an increase in the number of locules or, as Price and Drinkard (*14*) call them, cells. On the other hand, Currence (*1*) and MacArthur (*12*) refer to rough fruit without regard to locule number. Such confusion is easily accounted for by the fact that in the many-loculed fruits roughness is much more easily seen.

MATERIALS AND METHODS

The seed stocks used in this work were procured from the following sources: The department of genetics, Iowa State College; the department of horticulture, North Dakota Agricultural College; and commercial seedsmen. Fruit shape, i. e. polar and equatorial diameter ratio, was determined, unless otherwise stated, by the use of calipers. Locule number was determined by sectioning. Ovary measurements were made by the use of a low-power microscope and an eyepiece micrometer. Wherever possible, the mean of 10 fruits was used in determining fruit and ovary shape and locule number.

In this investigation the locule number will be dealt with, the suggested gene symbols *Lc* (few) and *lc* (many) locules being used, as distinct from the *Ff* (rough fruit or fasciation) genes.

For shape, *o* (oval) will be used to indicate a polar diameter 1.2 or more times the equatorial diameter, *O* (round) for a ratio of 0.95–1.2, and *O′* (oblate) for a ratio less than 0.95. *O′* is used for convenience only, inasmuch as the existence of this suggested allelomorph has not yet been proven.

Standard error rather than probable error is used. In all correlations, one asterisk (*) indicates a significant correlation and two asterisks (**) a highly significant one, as calculated from table 16 of Wallace and Snedecor (*17*).

The following known genes are involved in this study:

Dd	standard-dwarf plants.
Oo	oblate-oval fruits.
Ss	simple-compound flower cluster.
Rr	red-yellow fruit.
Yy	yellow-clear skin.
Spsp	indeterminate-determinate plant.
Cc	cut-potato leaf.
Aa	purple-green stem.
Ll	green-lutescent foliage.

EXPERIMENTAL PROCEDURE

SIZE, SHAPE, AND LOCULE NUMBER CORRELATIONS

Quantitative characters may often best be studied by determining whether they are associated with qualitative ones. It was decided to approach locule number in this way. Crosses were therefore made in which the parental stocks differed in locule number and in other characters which could be separated readily. F_2 and backcross progenies were then classified according to these, and their relation to mean locule number determined.

In table 1, under A, B, and C, such tabulated F_2 generations and backcrosses will be seen. In these it will be noted that there is a significant association between locule number and the first-chromosome-linked genes *Dd Oo Ss*, but no association with the *Rr* genes.

In B, the backcross population is divided into two classes *O* and *O'*, rather than *O* and *o*, because the plant used in test crossing was oblate, producing when crossed with *o* a round fruit, and when crossed with *O* an oblate fruit.

Populations D, E, F, and G show "dwarf" coming into the cross associated with many locules which is the reverse of the crosses in A, B, and C, but again there is no significant association with *Rr* and *Cc*. Population H involves *Yy*, and here again there is no evidence of association. This close association between genes of the first chromosome and locule number does not, however, prove the existence of any gene for locule number. Locule number might perhaps be an indirect effect of the shape gene, or of size previously reported as associated with first chromosome genes by Lindstrom (*10*).

TABLE 1.—*Association of certain tomato characteristics with locule number*

Populations [1] and parents (See p. 5)	Phenotypic segregates	Mean number of locules	Difference
A–F_2	197 *D*	3.21	0.70±0.09
$\frac{(26)\ D\ O\ S\ lc}{(3)\ d\ o\ s\ Lc}$	55 *d*	2.51	
	211 *S*	3.16	.52± .10
	41 *s*	2.64	
	134 *O*	3.33	.91± .08
	46 *o*	2.42	
B–backcross	28 *D*	4.61	.58± .11
$\frac{(26)\ D\ O\ R\ lc}{(3)\ d\ o\ r\ Lc} \times (46)\ \frac{d\ O'\ r\ lc}{d\ O'\ r\ lc}$	23 *d*	4.03	
	32 *O*	4.12	.44± .06
	19 *O'*	4.56	
	18 *R*	4.36	.02± .13
	33 *r*	4.34	
C–F_2	138 *O*	3.24	.68± .10
$\frac{(\text{Bison})\ O\ lc}{(\text{Yellow Pear})\ o\ Lc}$	44 *o*	2.56	
D–backcross	45 *D*	3.90	.57± .14
$\frac{(49)\ d\ lc}{(9)\ D\ Lc} \times (40)\ \frac{d\ lc}{d\ lc}$	64 *d*	4.47	
E–backcross	95 *D*	4.23	.49± .19
$(40)\ \frac{d\ O'\ r\ lc}{d\ O'\ r\ lc} \times \frac{(49)\ d\ O'\ r\ lc}{(9)\ D\ o\ R\ Lc}$	80 *d*	4.72	
	81 *R*	4.46	.01± .16
	94 *r*	4.47	
	96 *O*	4.26	.54± .13
	79 *O'*	4.80	
F–backcross	32 *D*	3.48	.61± .15
$\frac{(49)\ c\ d\ lc}{(10)\ C\ D\ Lc} \times (49)\ \frac{c\ d\ lc}{c\ d\ lc}$	33 *d*	4.09	
	34 *C*	3.85	.08± .15
	31 *c*	3.77	
G–backcross	59 *D*	2.45	.78± .11
$\frac{(9)\ D\ Lc}{(34)\ d\ lc} \times (49)\ \frac{d\ lc}{d\ lc}$	41 *d*	3.23	
H–F_2	103 *Y*	5.59	.09± .13
$\frac{(47)\ Y\ Lc}{(48)\ y\ lc}$	32 *y*	5.68	

[1] The capital letters A–H refer to populations; the numbers in parenthesis to the strains of tomatoes used.

As a means of studying the relationship of locule number to shape and weight of fruit, correlations were calculated between these characters in several F_2 and backcross populations. These are shown in table 2. Under populations A and B the larger parental varieties have the greater number of locules. The progenies in every case exhibit highly significant correlations between size and locule number; shape $\left(\frac{\text{equatorial diameter}}{\text{polar diameter}}\right)$ and locule number; and shape and weight. This is true even under A where the large many-loculed parental variety has oval fruits. No explanation for this is offered at this

point. It is mentioned merely to emphasize the point that there is a strong tendency for many-loculed fruits to be oblate.

TABLE 2.—*Correlations*[1] *between locules, weight, and shape in various* F_2 *and backcross populations*

Population and number of plants	Pedigree	Weight and locule number	Weight and shape	Locule number and shape
A-207	(Ohio Red) large, oval *lc* / (Yellow Cherry) small, round *Lc* F_2	**0.76	**0.30	**0.42
B-76	(Oxheart) large *lc* / (Yellow Cherry) small *Lc* F_2	**.51	**.37	**.47
C-28	(9) large *o Lc* / (36) small *O lc* F_2	.32	.06	**.85
D-47	(9) large *o Lc* / (36) small *O lc* × (40) large *O lc* / large *O lc*	.17	.00	*.31
E-15	(10) large *o Lc* / (36) small *O lc* × (40) large *O lc* / large *O lc*	.15	*.58	.46

[1] *=significant correlation; **=highly significant.

Data from crosses between comparatively large tomatoes with few locules and smaller many-loculed ones would seem to afford some possible clue to the causal relationship between size, shape, and locule number. Small, many-loculed varieties not being available an attempt was made to produce them. Crosses were therefore made between Bison, a large, many-loculed oblate tomato, and Red Currant, a few-loculed, round one; and also between Bison and Yellow Cherry. These, carried through several generations, did give pure oblate strains with many locules which had smaller fruits (42 g.) than those of the largest two-loculed oval varieties (56 g.). The synthesis of such a line indicates that locule number is not a mere secondary effect of size. These synthesized lines did not, however, have smaller locules than those of the small two-loculed parent but carried a larger number of similar sized locules and the fruits were therefore not as small. Likewise, the large two-loculed variety, while very large for a two-loculed sort, did not equal the weight of the common, many-loculed, large fruits except in weight per locule.

Under C in table 2 are found the F_2 results of the cross between a small, many-loculed and a large, few-loculed tomato. In determining shape correlations the ratio of $\left(\frac{\text{equatorial diameter}}{\text{polar diameter}}\right)$ was used as a a shape index, with classes from 0.60 to 1.80 at 0.10 intervals. Under D and E are found the F_1 results of such a cross backcrossed to large, many-loculed forms. It may be noted that while correlations exist, they are small in the case of weight and shape, except under E where the population is small and the correlations are not highly significant. The correlations between locules and weight are likewise not significant under C, D, and E where the smaller parent is many-loculed. This is in contrast to A and B where the large-fruited parent is many-loculed and where such correlations are highly significant.

Partial correlations may be used in studying the relation between size, shape, and locule number. Such correlations as those presented in table 3 show that when the parental *lc* stock is the smaller-fruited the correlation between shape and locule number is practically

unchanged if weight be eliminated; that the correlation between locules and weight likewise remains about the same when shape is eliminated, but that the correlation between weight and shape becomes negative when locule effect is removed, thus indicating that weight and shape are associated because of their mutual association with locule number. These data throw much doubt on the possibility of oblate shape being the cause of large size. A possible hypothesis would be that the small locules in the oblate parent tend to remain associated with oblateness in the progenies and to cause the oblate tomatoes in the F_2 and test crosses to be smaller, while the many locules carried by the same parent tend to increase the size of tomatoes possessing this character.

Lindstrom (*8*) concluded that locule number did not appreciably influence fruit weight, with equatorial diameter held constant. Since, however, equatorial diameter itself is a measure of size and perhaps also partly the effect of locule number, the shape index is used in this study instead of equatorial diameter. While the data of table 3 show significant correlations between locule number and weight, these are not so large as those between locule number and shape. This phenotypic association of locule number and shape has led to the work on the inheritance of locule number set forth later in this paper.

TABLE 3.—*Simple and partial correlations between* [1] *number of locules, weight and shape in F_2 populations and backcrosses involving small oblate many-loculed and large oval 2-loculed tomato varieties*

Population, number of plants	Pedigree	Locules and shape	Locules and shape (weight eliminated)	Locules and weight	Locules and weight (shape eliminated)	Weight and shape	Weight and shape (locules eliminated)
A-87	$\frac{(10)\ \text{large}\ Lc\ o}{(36)\ \text{small}\ lc\ O'}$ F^2	**0.84	**0.82	**0.47	**0.41	**0.31	−0.16
B-44	$\frac{(9)\ \text{large}\ Lc\ o}{(35)\ \text{small}\ lc\ O}$ F^2	**.65	**.55	**.68	**.59	**.41	−.04
C-28	$\frac{(9)\ \text{large}\ Lc\ o}{(36)\ \text{small}\ lc\ O}$ F^2	**.85	**.91	.32	**.70	−.06	**−.65
D-203	$\frac{(9)\ \text{large}\ Lc\ o}{(36)\ \text{small}\ lc\ O} \times (50) \frac{\text{large}\ lc\ o}{\text{large}\ lc\ o}$	**.70	**.79	**.49	**.65	.03	**−.50
E-70	$\frac{(10)\ \text{large}\ Lc\ o}{(35)\ \text{small}\ lc\ O} \times (50) \frac{\text{large}\ lc\ o}{\text{large}\ lc\ o}$	**.88	**.92	**.62	**.69	**.36	**−.50
F-47	$\frac{(9)\ \text{large}\ Lc\ o}{(36)\ \text{small}\ lc\ O} \times (40) \frac{\text{large}\ lc\ O}{\text{large}\ lc\ O}$	*.31	*.31	.17	.18	.00	**−.60
G-58	$\frac{(15)\ lc\ o}{(51)\ Lc\ O} \times (15) \frac{lc\ o}{lc\ o}$	**.79	**.79	**.69	**.69	**.38	**−.85

[1] See footnote 1, table 2.

Figure 1 depicts the distribution of F_2 and backcross populations from crosses between few-loculed and many-loculed plants. In both cases there are bimodal distributions with the low point falling at 3.5 locules. This, together with the fact that in crosses between few-loculed varieties and many-loculed ones the F_1 is two- to three-loculed, suggests this as a natural point of division between *lc* (many-loculed) and *Lc* (few-loculed). In the case of the F_2, approximately three-fourths (133) of the population would thus be classed as *Lc* and one-fourth (49) as *lc*, which fits a Mendalian 3:1 ratio with $P=0.74$. In the backcrosses the division of the population above and below this point is in the proportion of 1:1, namely 84:83. A single major gene for locule number is therefore proposed.

If locule number is due to a gene *lc*, the production of an *o lc* tomato should be possible. Steps were taken to synthesize such a variety and the outcome was successful. In the meantime a pure variety (no. 15) evidently having the same combination was selected from a strain from J. W. Lesley of California. This was used because it also carried the recessives *d* and *s*. This form, carrying genes for oval with many locules, had phenotypically round fruit, according to hypothesis, because the added locules offset the phenotypic effect of *o*. If this tomato were crossed with one carrying *Lc* and *O*, the F_2 should, if the hypothesis is correct and linkage not too great, give some plants which would be *o Lc*, and the fruit therefore oval. This procedure was followed and oval-fruited plants appeared as expected.

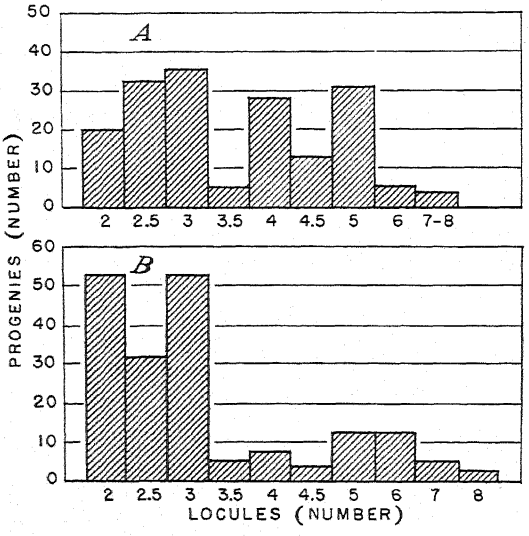


FIGURE 1.—Segregation of progenies according to locule number. Of the parental stocks used Sunrise, Yellow Pear, and Red Pear were two- to three-loculed, 15 and Bison were four- to five-loculed. Locule numbers were obtained by securing the means of 10 fruits on each plant which accounts for plants classified as having 2.5 locules per fruit, etc.

A, Combined $\frac{15}{\text{Sunrise}} \times 15$ and $\frac{15}{\text{Red Pear}} \times 15$ (backcrosses);

B, $\frac{\text{Bison}}{\text{Yellow Pear}}$ F_2.

A backcross between the F_1 and *dd, oo, ss, lclc* gave similar results. Here oval, two-loculed tomatoes appeared from parents both of which were phenotypically round.

Assuming that the round, many-loculed tomato (no. 15) did carry *oo* but, because of many locules was phenotypically round, and that many locules are recessive to few, a cross between it and an *LcLc oo* tomato should give in the F_1 not a round but an oval, few-loculed fruit. Such a cross was made and it fulfilled expectations. The F_2 and backcross populations disclosed no 2-loculed, round-fruited plants nor any many-loculed ovals, but as the number of locules increased the shape changed to oblate.

Data have already been presented in table 1 which suggest linkage between locule number and first-chromosome genes. Table 4 gives data showing the amount of this linkage with *Ss* and *Dd*. These furnish a basis for the location of *Lclc* on the chromosome map. Each population shows linkage between *Lclc* and *Ss* with a mean cross-over percentage of 20.5. Between *Lclc* and *Dd*, however, there is a mean cross-over percentage of 46.9. From these data it would seem safe to suggest that there is a gene for increased locule number (*lclc*) linked with one for compound cluster (*ss*) and that its locus is on the opposite end of the chromosome from *Dd*. With a 20.5 percent cross-over value between *Lclc* and *Ss*, as calculated here, and more than 30 units between *Dd* and *Ss* according to MacArthur, the linkage between *Dd* and *Lclc* should be very slight, as the figures in table 4 show

TABLE 4.—*Linkage relations of lc to s and d*

Parentage [1]	Progeny				Cross-over value	X^2 2rn	P [2]
	S Lc	*S lc*	*s Lc*	*s lc*			
$\frac{(52)\ S\ Lc}{(15)\ s\ lc}\ F_2$	48	3	5	9	0.142±0.026	25.7	0.01—
$\frac{(Sunrise)\ S\ Lc}{(15)\ s\ lc}\times(15)\ \frac{s\ lc}{s\ lc}$	42	12	14	36	.250± .034	29.3	.01—
$\frac{(Sunrise)\ S\ Lc}{(15)\ s\ lc}\ F_2$	102	24	15	27	.248± .039	37.89	.01—
$\frac{(26)\ S\ lc}{(3)\ s\ lc}\ F_2$	152	59	37	4	.325± .056	6.02	.02
$\frac{(Red\ Pear)\ S\ Lc}{(15)\ s\ lc}\times(15)\ \frac{s\ lc}{s\ lc}$	23	5	4	25	.155± .043	27.5	.01—
$\frac{(Red\ Pear)\ S\ Lc}{(15)\ s\ lc}\ F_2$	104	5	10	20	.108± .028	61.47	.01—

Parentage	Progeny				Cross-over value	X^2 2rn	P
	D Lc	*D lc*	*d Lc*	*d lc*			
$\frac{(Sunrise)\ D\ Lc}{(15)\ d\ lc}\times(15)\ \frac{d\ lc}{d\ lc}$	30	24	26	23	0.478±0.05	0.063	0.80
$\frac{(Sunrise)\ D\ Lc}{(15)\ d\ lc}\ F_2$	91	43	26	8	.561± .05	1.313	.25
$\frac{(26)\ D\ lc}{(3)\ d\ Lc}\ F_2$	137	60	52	3	.240± .03	9.820	.01—
$\frac{(Red\ Pear)\ D\ Lc}{(15)\ d\ lc}\times(15)\ \frac{d\ lc}{d\ lc}$	14	22	14	8	.620± .06	3.566	.06
$\frac{(Red\ Pear)\ D\ Lc}{(15)\ d\ lc}\ F_2$	82	16	32	9	.448± .06	.322	.60

[1] Number in parentheses refers to strain numbers.
[2] *P* value indicates the probability of differences being due to chance.

OVAL FRUITS NOT ALWAYS DUE TO THE *o* GENE

In the study of inheritance of size and shape the Ohio Red variety was used because it is large in size, nearly oval in shape, and each fruit carries several locules. One of the first crosses made with this variety was with a tomato carrying *dd rr oo LcLc*. In the F_2 from such a cross 64 plants among a total 256 were classed as oblate, and 75 as round. The appearance of oblate-fruited plants in the F_2 in crosses between these two oval varieties would indicate that the two ovals are not due to the same gene. Inasmuch as there was some possibility that the Ohio Red used in this cross was not genetically pure, another cross was made between Yellow Plum and a new stock of Ohio Red secured from the Department of Genetics of Iowa State College which had been carried through several generations and found pure. Here again 6 of the 47 plants carried decidedly oblate fruits and 5 were round.

Further evidence for the belief that the large oval differs from *o* is found in the fact that while crosses between *oo* tomatoes and *OO* two-loculed varieties produce an oblate F_1, Ohio Red in such cases gives an intermediate slightly oval $\left(\frac{L}{W}=1.1\right)$.

In F_2 populations it was found impossible to fit the progeny to a simple Mendelian hypothesis. Multiple factors are therefore

suggested. Attempts to detect linkage with the genes *Dd*, *Rr*, *Yy*, *Aa*, *Ll*, *Cc*, and *Spsp* failed, with the exception of *Dd*, and even this was inconclusive, and may have been due to the association of *Dd* with differences in locule number present in the parent stocks.

THE DEVELOPMENT OF SHAPE

In connection with the study of the inheritance of shape and size in tomatoes, measurements were made of the length and width of the ovaries at the time of anthesis, and these were compared with similar measurements of mature fruits from the same plants. The correlations between such measurements are shown in A of table 5 for an F_2 population of a cross between Yellow Pear, small-oval; and Bison, large-oblate. B gives similar figures for a group of plants of many varieties, excluding Ohio Red and Oxheart. C shows a cross involving Ohio Red. The correlation between the shape of ovary and the shape of the mature fruit in A and B is very striking, so great in fact that it seems probable that with ordinary varieties such measurements might be a better indication of the actual genetical constitution of the plant than the measurement of mature fruits, since the amount of seed and growing conditions have considerable effect upon the shape of the fruit when mature.

TABLE 5.—*Correlations between ovary measurements at anthesis and mature fruit measurements*

Population	Plants	Ovary and fruit correlations [1]		
		Length	Width	Ratio $\frac{L}{W}$
	Number			
A — $\frac{\text{(Yellow Pear) } o}{\text{(Bison) } O}$ F_2	179	**0.594	**0.953	**0.923
B — Miscellaneous varieties	78	**.619	**.639	**.903
C — $\frac{(53) \quad O}{\text{(Ohio Red) large-oval}}$ F_2	61	.038	**.694	**.598

[1] See footnote 1, table 2.

These data are in line with those of Hackbarth et al. (*3*) who found a high degree of correlation between size of tomato ovaries and mature fruits. Houghtaling (*5*) explained this as the result of a differential growth rate which exists from earliest primordia until anthesis by which time cell division is completed. Increase in size thereafter is due to an increase in cell size. Contrasted to this is the condition in *Cucurbita* as reported by Sinnott and Kaiser (*16*), where differences in fruit shape exist at earliest primordia, and in *Capsicum* where differences appear after anthesis. A condition similar to that in *Cucurbita* was also reported in cucumbers by Hutchins (*6*) and in watermelons by Weetman (*19*).

With large-oval it will be noted (C in table 5) that length correlation is not significant. Measurements of the developing ovaries of Ohio Red revealed that at anthesis the ovary is round but that length growth proceeds faster than width until the fruit attains one-third its full length with the result that the mature fruit is oval (fig. 2, *I*). The

inference is that the differential growth rate which Houghtaling (*5*) found to cease at anthesis continues much longer in this case. Histological studies showed that cell division does continue thus after anthesis. This is more evidence that large-oval is not the result of the *o* gene.

THE DEVELOPMENT OF PEAR SHAPE

Pear shape has been something of a puzzle since the earliest genetical work with tomato. It was first used as the designation for lengthened fruit shape but was later replaced by "oval." While an examination of developing ovaries reveals that oval shape may be detected long before anthesis, pear shape is often not evident until near blooming time. At anthesis in pear-shaped varieties, the ovaries have the appearance of being molded into pear form by the constricting effect of the cone forming corolla tube around the pistil. Longitudinal sections at various times show that the seed cavities extend internally into the base of the ovary until the external, necked appearance is visible under a hand lens (fig. 2–*A*, *B*, *C*).

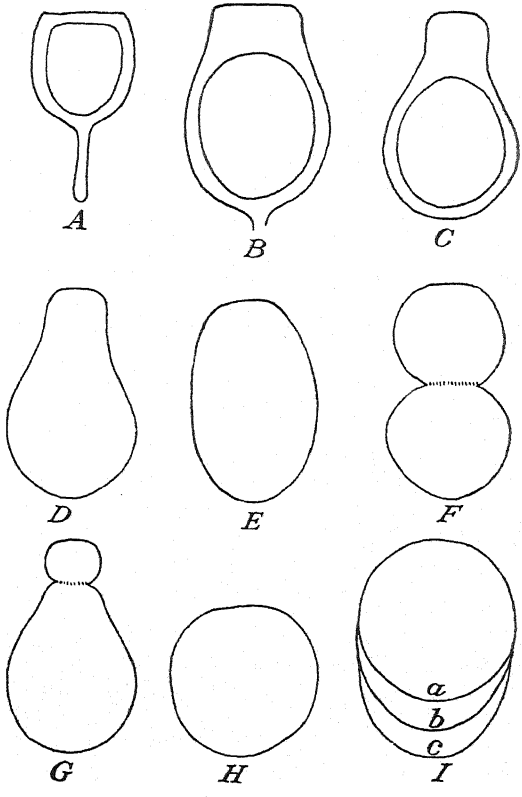


FIGURE 2.—Development of pear shape: *A*, Some time before anthesis; *B*, at anthesis. *C*, Mature fruit; note shift in location of locule. *D*–*G*, Fruit shape variations to be found on the same plant, *F* and *G* undoubtedly having been caused by persisting corolla tube constriction. *H*, Fruit resulting from a blossom of the pear variety from which the corolla tube was removed some time before anthesis. *I*, Shape changes in large oval tomatoes such as Ohio Red: *a*, At anthesis; *b*, blossom faded; *c*, from one-third mature fruit length to maturity.

Genetically identical stocks raised in different environments may vary in the degree of pear shape in mature fruits. For example, two groups of plants of a Red Pear variety were raised, one in the field and the other in the greenhouse. The crop in the field was typically pear-shaped, the one inside had few distinctly pear-shaped fruits. Even on the same plant some specimens may be markedly pear-shaped and others oval but not pear-shaped. Occasionally fruits may be grooved in the middle or with the neck of the fruit showing an added constriction. When these were first seen on plants in commercial breeding blocks the plants were discarded as being rogues, but, an examination disclosed remnants of the corolla tube. This suggested that pear shape might be induced by the fused corolla tube. If this is so, an early removal of the corolla should result in the production of fruits without a neck. Such an operation on blossoms of the pear-shaped tomato did produce this effect in some cases as will be noted in

figure 2, *H*. Pear shape may therefore be a secondary effect of a constricting corolla. This does not mean that this tendency is not inherited, but it does throw some light on the reason its inheritance is difficult to study. Not only was pear shape not evident in some fruits developing from flowers the corolla tubes of which were removed early, but in several instances, such as the one shown in figure 2, *H*, the resulting fruit was round rather than oval, suggesting that oval shape itself may be an induced condition.

Typical pear-shaped fruits were also produced artificially by constriction during the period of cell division by the use of a thread tied around the blossom buds of ordinary plum-shaped tomatoes at an early stage and removed at anthesis.

DISCUSSION

Any study of the inheritance of fruit size and shape in the tomato plant reveals that the matter is complex. Environment is well known to affect both characters. The vegetative vigor of a plant may greatly affect the size of a fruit. This being true, genes not directly related to size and shape of the fruit in such a plant as the tomato but which affect its general vigor and other plant characteristics, as suggested by MacArthur (*12*), have their effect. When genetically different plants are grown where there is variation in external conditions, such as temperature, light, soil, and parasites, the effect of the different genes will be modified. Even on the same plant, conditions differ enough in different parts to affect size and shape.

During the 6 years of this investigation many tomato crosses were made and studied in an attempt to learn more of the development and inheritance of fruit shape and size. It was found that there is a high degree of association between locule number, size, and shape, and that an association exists between qualitative first chromosome factors and locule number. By means of partial correlations the existing correlations between shape and weight were shown to be due at least in part to their mutual correlation with locule number. Locule number was found to segregate in a ratio of 3:1 of few to many-loculed plants in F_2 populations, and in a ratio of 1:1 in backcrosses, the dominant group carrying two to three locules, while the recessive group carries three and one-half and more locules. A round tomato with many locules has been shown to be *oo lclc* by means of crosses with two-loculed round tomatoes and two-loculed oval tomatoes. Linkages with first chromosome genes were calculated which demonstrate that the *Lclc* locus is on the opposite end of the chromosome from *Dd* beyond the locus of *Ss*. The presence of this gene for locule number may have an effect on size of fruit by an increase or decrease in the number of locules without affecting size of locule. This relationship is not fully demonstrated and even if it were it would not account for all size differences. Obviously there are other size genes, since oval two-loculed tomato varieties are known which differ from each other by several hundred percent. The same is true of round two-loculed sorts. More work is needed on the locule number and size relationship.

The existence of the genes *Lclc* is of great importance in accounting for variations in tomato shape. The genes *Oo* which affect gross fruit shape through their effect on locule shape together with *Lclc* which affect shape by varying the locule number, provide a key for explaining gradations as follows: *OLc*—round, *Olc*—oblate, *oLc*—oval, and *olc*—round.

Correlations between the ovary and the fruit have confirmed the findings of others that with most varieties the size and shape of tomatoes are rendered predictable at blossoming time by measuring the ovaries. Observations have shown marked effects of environment on pear shape. Mature fruits have been observed in which a persistent corolla tube has obviously caused constrictions. Early removal of the corolla has resulted in fruits without pear shape on plants where control blossoms produced pear shape. These suggest that pear shape is probably induced by the constricting effect of the fused corolla tube.

The importance of such developmental studies is emphasized by the differentiation of two groups of oval tomatoes recorded in this paper. The one type symbolized by *o* is composed of the usual comparatively small-fruited varieties in which the final shape and size is fixed at blossoming. The other, including the larger-fruited varieties, may due to a complex that causes continued elongation. When this is superimposed on the genetic complex producing a round tomato, it becomes oval, and upon an oval tomato, extreme length is the result; but when added to an otherwise oblate tomato the result is round. The nature of this complex and its inheritance has not been cleared up. In order to clarify this it would seem desirable to use only *LcLc* varieties for both parents. This would require either the discovery of, or the synthesis of, a pure elongated *LcLc* stock which might require considerable time and labor. If this were done it is possible that the existence of a single major gene for continued elongation could be demonstrated. Such confusion as has been observed to date may be only the effect of *Oo*, *Lclc*, and perhaps other recognized genes such as *Ff*.

Practical breeding work has demonstrated the possibility of recombining *Oo*, *Lclc*, and the factors for elongation. Comparatively large, very oval, few-loculed strains from Iowa State College, when crossed with Early Jumbo, a large oblate, have resulted in lines closely resembling Oxheart. Oxheart, a large, many-loculed heart-shaped fruit, when crossed with Yellow Cherry, resulted in segregates that were very oblate, small, and many-loculed. Ohio red crossed with Yellow Pear has given extremely long, few-loculed segregates. While these examples are not offered as a definite proof of the existence of a single gene for added elongation they do point toward no more than a rather simple complex.

CONCLUSIONS

1. There is significant correlation between shape, size, and locule number in tomato fruits. Part of the correlation between shape and size is due to their mutual correlation with locule number.

2. A major gene for locule number exists. Two to three locules, *Lc*, is dominant over three and one-half or more locules, *lc*.

3. The locus of *Lclc* is on the first chromosome beyond *Ss* with about 20 percent cross-over.

4. The effect of *lc* when associated with *o* is to produce a phenotypically round tomato.

5. With most of the tomato varieties, the size and shape of the matured fruit is predictable from ovary measurements at anthesis.

6. Pear shape is probably due to the constricting effect of the fused corolla tube.

7. Oval tomatoes in some cases are the result of genes other than *o*, the effect of which is to continue the elongation of the ovary after anthesis.

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AN UNDESCRIBED POTATO DISEASE IN WEST VIRGINIA[1]

By C. R. ORTON, *plant pathologist*, and L. M. HILL, *graduate assistant in plant pathology, Department of Plant Pathology and Forestry, West Virginia Agricultural Experiment Station*

HISTORY

In 1931 a disease was observed on a few scattered plants of potato (*Solanum tuberosum* L.) in Preston County, W. Va. Since then it has spread steadily and has become a limiting factor in potato production in certain districts of the State. This disease has been observed also in Pennsylvania and Maryland. Altitudes, soil types, and moisture conditions have not appeared to be significant factors in the distribution or the intensity of this disease, nor does it appear to be influenced by fertilizer treatments.

All strains of Rural, both russet and smooth, are very susceptible. The Irish Cobbler strains and Green Mountain, Early Rose, Chippewa, Katahdin, and other varieties grown in West Virginia have also been attacked. The early varieties are less frequently diseased, possibly because they mature before the peak of infection is reached.

The origin of the disease is unknown. Some observers believe that it has been present for 12 years or more. The importance of the disease with respect to yield depends largely upon the stage of development of the potato plant at the time of infection. One field which showed nearly 100 percent severely infected plants in 1935 yielded less than 10 bushels of United States No. 1 tubers per acre. Similar cases were recorded in 1936.

DIAGNOSIS

MACROSCOPIC SYMPTOMS

The first external symptoms are a dwarfing of the upper stem, accompanied by a folding upward and narrowing of the terminal leaflets (pl. 1, *A*). This is soon followed by a paling of the foliage, and in Rural varieties by the appearance of a purplish tinge on the margins of the younger leaflets. Soon the rest of the foliage fades, and in the Rural varieties the stem assumes a purplish color. Wilting follows rapidly and the plants make an attempt to recover by sending out axillary shoots which may reach a length of 3 to 6 inches. These shoots present symptoms similar to those observed on the primary terminals. They are short-lived, and the whole plant dies a week or 10 days later. Aerial tubers have been observed rather frequently but may be the result of other causes.

The roots have a dull color, are reduced in size and length, and break easily when the plants are pulled. Tuber production is dependent upon the time of infection. Plants which become infected before tuberization produce few if any tubers of marketable size. Only the plants which become infected at about the time of maturity may pro-

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duce a normal yield. The intermediate periods of infection induce corresponding effects upon yield.

While the time of infection and the period of incubation have not been determined, it is known that the plants generally die within 2 weeks after the appearance of the first unmistakable symptoms.

The internal macroscopic symptoms which are of the greatest diagnostic value are as follows: A general brownish discoloration of the vascular region which begins at the base of the stem prior to the appearance of foliage symptoms, and progresses through the roots, stem, and stolons into the tubers, and an occasional rusty spot which is usually present in the lower pith region of the stem and often throughout the stem pith. Only the cortical region shows necrosis in cross or in longitudinal sections of the roots. The stolons present a picture similar to that of the stem. Necrosis in the tubers is very characteristic. There is a pronounced and intense brown discoloration at the point of rhizome attachment. From this region inward a branched system of necrotic woody strands extends through the vascular region, involving one-quarter to one-third of the entire vascular system. At the same time numerous strands, often dendritic in appearance, extend less deeply into the internal storage parenchyma. A transverse section of the tuber just inside the point of stolon attachment discloses a picture similar to that found in net necrosis, although it is less extensive in the internal parenchyma (pl. 1, *B*, *C*). The tubers do not rot in storage unless invaded by secondary organisms.

MICROSCOPIC STUDIES

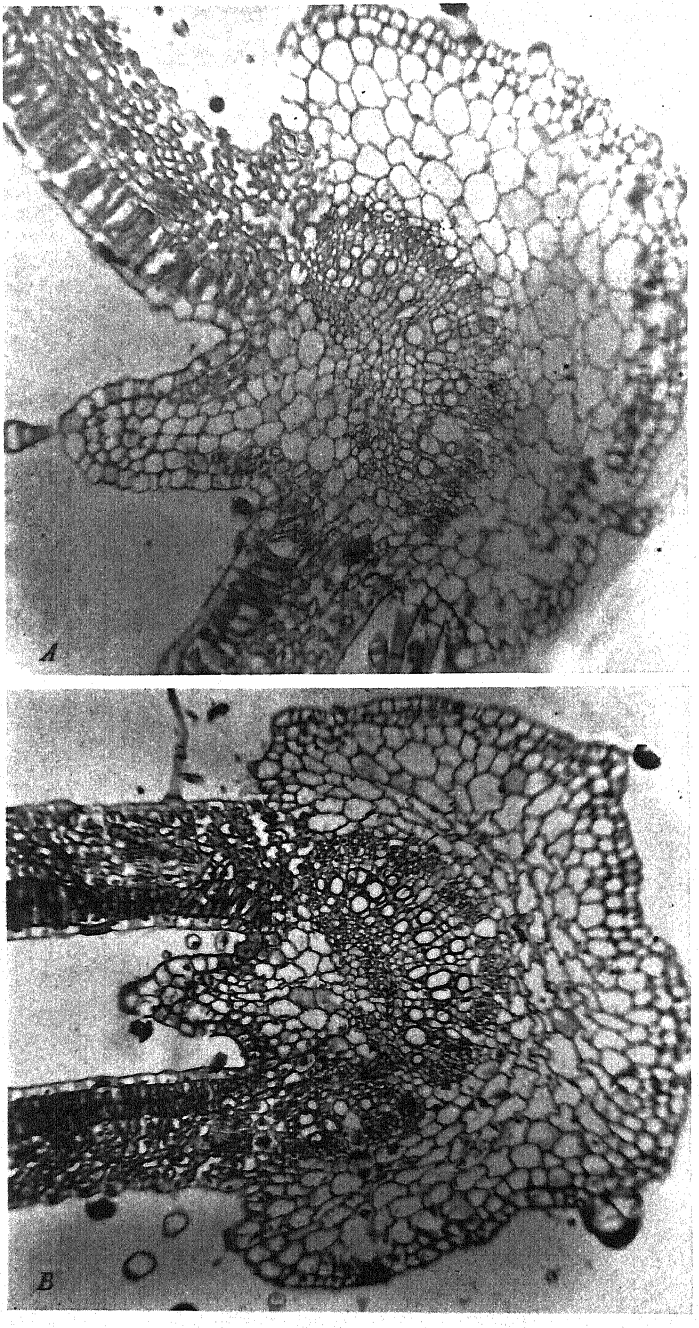
The material for study in 1935 was obtained from a commercial field of Russet Rural in Preston County. This field was relatively free from all other diseases. The material was fixed in formol-acetic-alcohol, and the usual methods of dehydrating and embedding in paraffin were used. Stem sections were cut 15 microns and all other parts 10 microns in thickness. Flemming's modified triple stain was used exclusively. The material for study in 1936 was obtained from the descendants of the foregoing crop of 1935. In addition to this, specimens from caged and uncaged healthy plants were obtained for comparison. All sections were cut 10 microns in thickness.

The most conspicuous anatomical feature to be noted in the young leaflets is the marked changes which take place in the palisade and mesophyll tissues (pl. 2, *A* and *B*). While the palisade cells in the diseased leaflets do not reach normal size, the most remarkable change is brought about by the laterally crowded condition of these cells and by the consequent closing of the large intercellular spaces which are so evident in healthy palisades. A secondary palisade is frequently found in the diseased leaves. Similar changes take place in the spongy mesophyll, where the intercellular spaces are greatly reduced by the crowding of the cells. The effect of these changes is reflected in the narrowing of the lamina, but no marked reduction in thickness has been seen. There is no appreciable change in the tissues of the petiole if necrosis is not present.

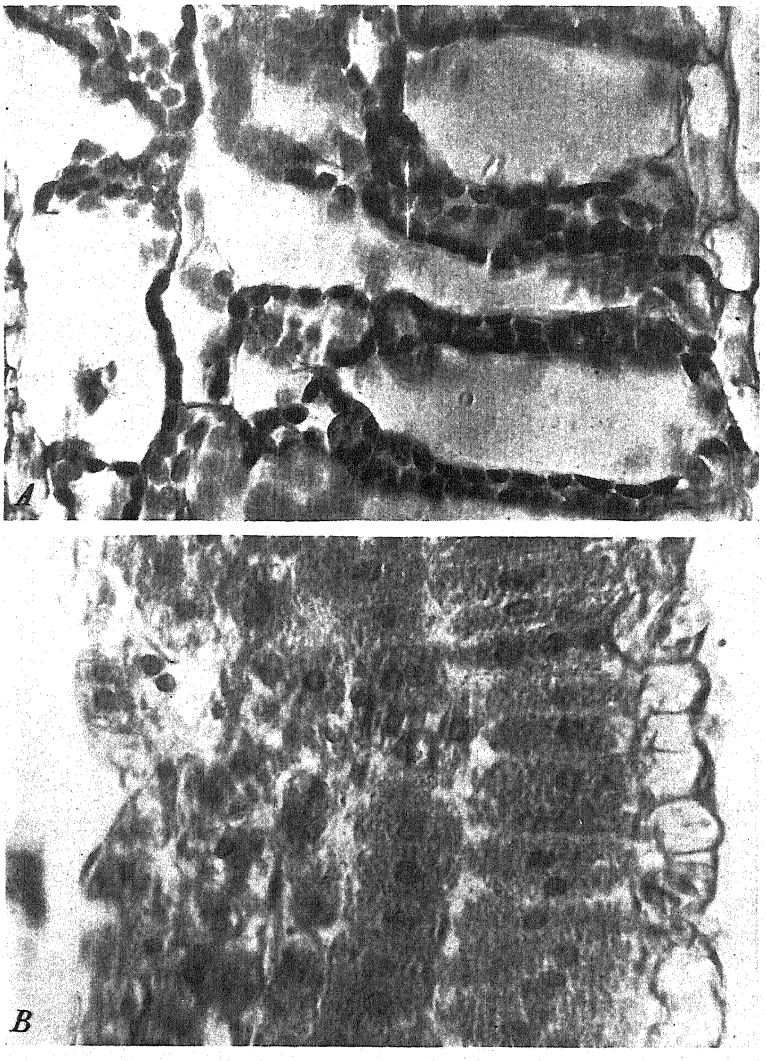
These changes are accompanied by a depletion of the chloroplasts during which they lose their starch as well as their normal vacuolated appearance. Consequently they appear empty or contain only a few stainable threads and soon they lose their regular outline, collapse,



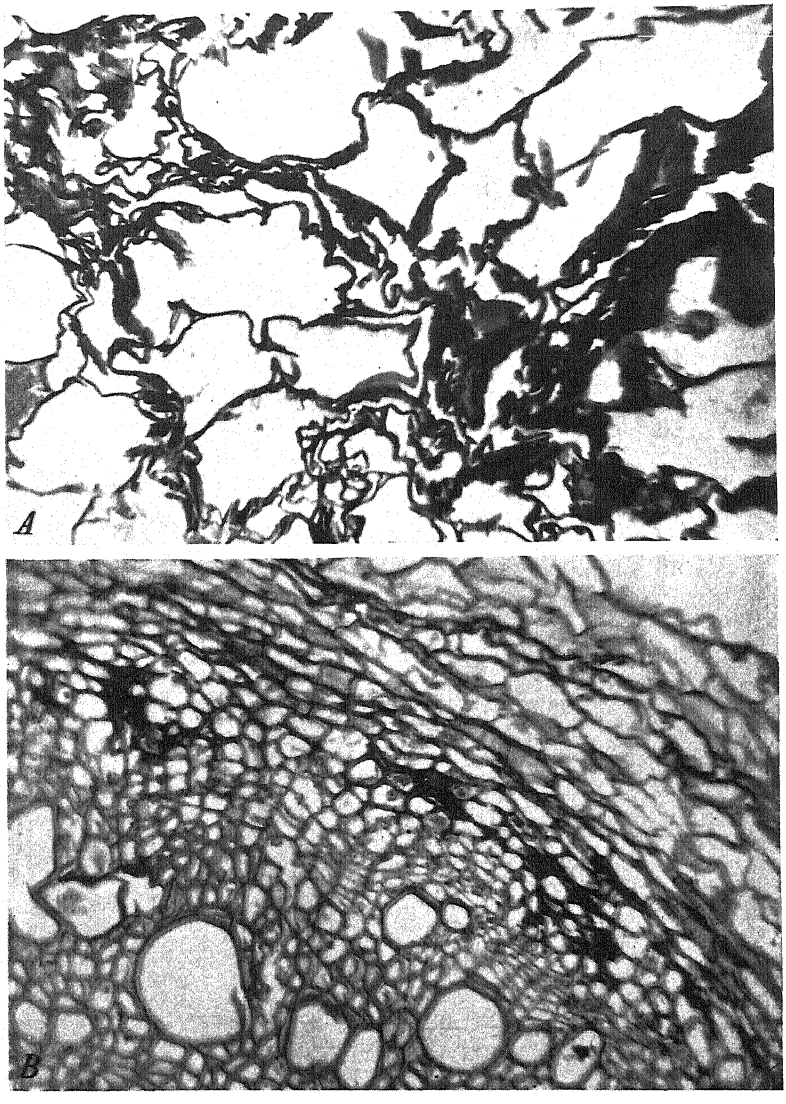
A, Late symptoms of a diseased Russet Rural potato plant showing upward folding of terminal leaflets and development of axillary shoots. *B*, Transverse section of stem end of tuber showing necrosis. *C*, Longitudinal section of tuber showing necrosis in vascular region and in the fundamental tissues.



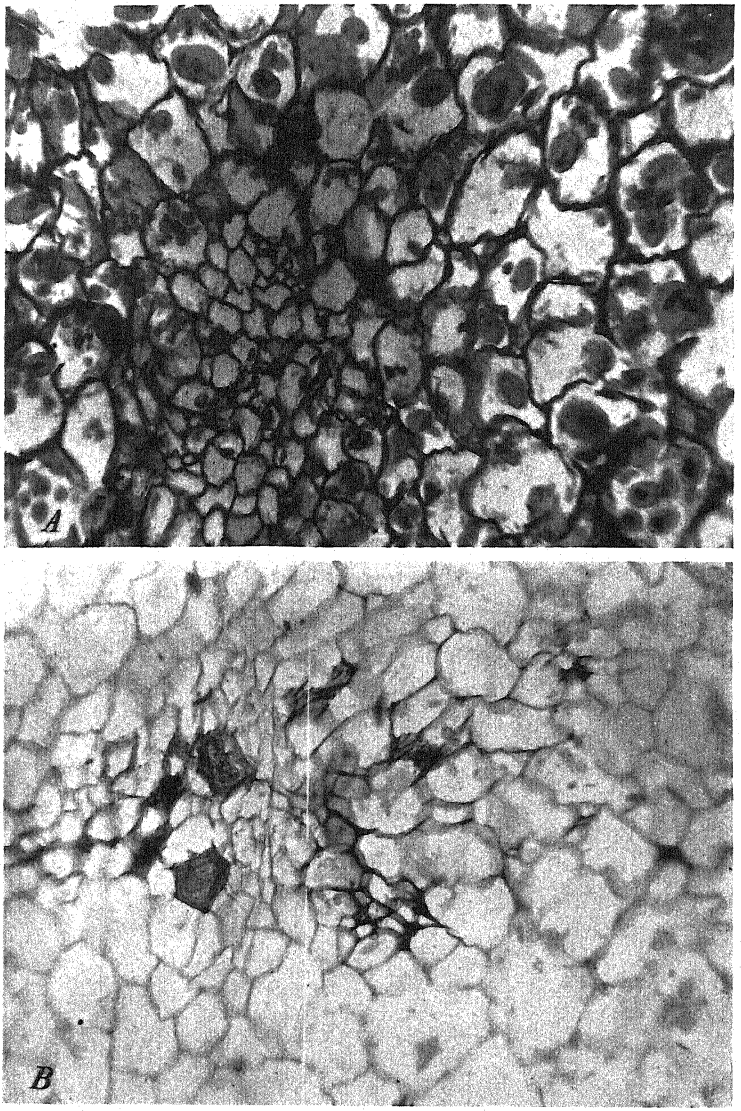
A, Transverse section of healthy petiole and laminae showing intercellular spaces in palisade tissue; note the angle of attachment of the laminae to petiole. × 130. *B*, Transverse section of diseased petiole and laminae showing absence of intercellular spaces in palisade; prominent nuclei throughout palisade; and the sharp angle of attachment of laminae to petiole. × 130.



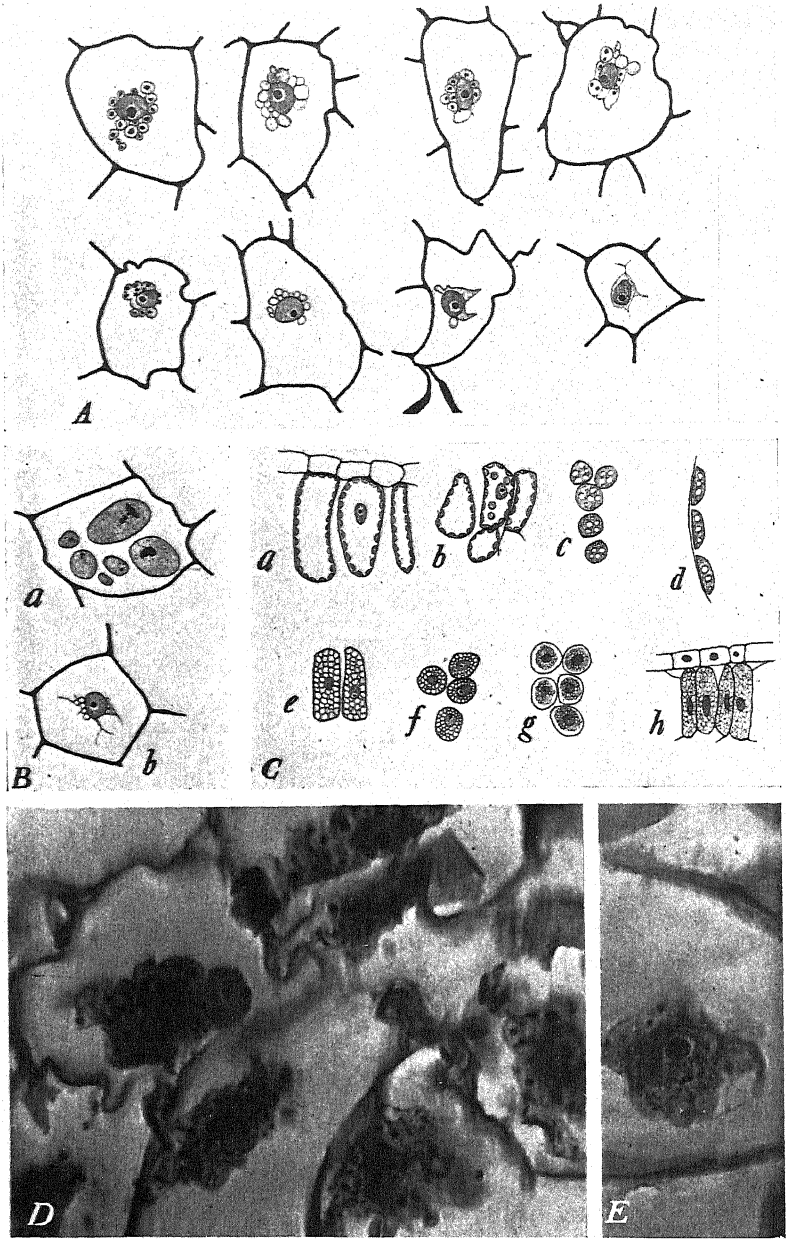
A, Healthy lamina; note large intercellular spaces and normal shape and position of chloroplasts. × 750. *B*, Diseased lamina showing absence of intercellular spaces; prominent nuclei; secondary palisade; and depleted chloroplasts which are diffused throughout the cells. × 750.



A, Transverse section of pith necrosis in lower stem with collapsed cells and granular deposit. × 130.
B, Transverse section of root showing phloem and cortical necrosis. × 240.



A, Transverse section through phloem of healthy tuber showing normal storage of starch grains. × 250.
B, Transverse section of diseased tuber showing phloem, xylem, and parenchyma necrosis with vessel filled with granular mass; note absence of starch grains adjoining necrotic areas. × 250.



A, Progressive stages in starch dissolution. The black center of small spheres is the hilum which disappears in late stages of dissolution. *B. b*, Final stage of starch dissolution leaving a threadlike mass around nucleus; *a*, healthy cell with normal starch grains. × 275. *C, a*, Normal palisade cells, × 275; *b*, normal mesophyll cells, × 275; *c*, normal chloroplast in mesophyll, × 980; *d*, normal chloroplasts in palisade, × 980; *e* and *f*, palisade and mesophyll cells, intermediate stages of depletion of chloroplasts. Vacuoles have disappeared leaving chloroplast membranes (in palisade and mesophyll cells) × 375; *g*, mesophyll cells, late stage of chloroplast depletion with remains grouped around nuclei, × 375; *h*, palisade cells, late stage of chloroplast depletion with remains scattered throughout palisade cells; nuclei prominent, × 375. *D*, Intermediate stage of starch dissolution showing starch grains aggregated around nuclei. × 980. *E*, Late stage of starch dissolution showing a group of spheres around nucleus. × 980.

leave their parietal position, and become scattered throughout the cell (pl. 3, *A*, *B*). In the mesophyll cells the depleted chloroplasts have a marked tendency to cluster about the nucleus (pl. 6, *C*, *g*). In later stages of disorganization they stain more deeply than the normal chloroplasts.

At this stage of disorganization the nucleus undergoes a marked change. The nuclear membrane takes a much darker stain, appearing nearly black and the chromatin loses its affinity for the gentian and becomes masked with safranin so that the nucleolus is differentiated only as a denser spherical body. The hyaline zone which is conspicuous about the nucleolus under normal conditions becomes masked by the dense safranin stain (pl. 6, *C*, *e*, *f*, *g*, *h*). The darker staining nuclei become more prominent and form a dark line which is conspicuous throughout the palisade (pl. 2, *B*).

Phloem necrosis is one of the early microscopic symptoms in the stem and appears first in the basal region. The necrosis may be confined to one phloem group, either internal or external, but as the disease progresses it appears in other phloem groups where the sieve tubes and companion cells are most generally involved. The necrosis also extends into the adjacent fundamental tissue. With the triple stain these necrotic areas become sharply marked through the intense safranin stain taken up by the walls of the cells involved. As soon as the phloem necrosis is well developed, a granular mass appears which may fill the dead cells and in some cases also a large part of the lysigenous space which frequently develops in the necrotic area. The mass is made up of granules of various sizes and shapes so densely crowded that their structure is obscure (pl. 5, *B*). In unstained sections this mass is yellow; in sections stained by the triple method, the mass stains heavily with safranin. The necrosis of both the internal and external phloem tissues develops in a discontinuous manner throughout the stem, the secondary shoots, the stolons, the stem end of the tuber, and the protophloem of the roots, where it becomes very extensive. This discontinuous development of the phloem necrosis is readily demonstrated in longitudinal sections, where the necrotic areas show a considerable variation in extent.

Examination of stained transverse sections of the xylem from diseased plants does not show the marked effect which would be expected from the extensive browning of the tissues. Most of the xylem takes the normal safranin stain except for an occasional vessel or small group of vessels lying near the cambium, the walls of which take a darker safranin. Such cells invariably contain a finely granular mass quite different in appearance from the masses found in the phloem. Those in the xylem are more spongy, frequently cytoplasmic in appearance, and take a bright gentian stain, in contrast to the safranin affinity shown by the masses associated with necrosis in the phloem. In some cases these inclusions in the vessels are so dense as to appear to occlude the cell; in other cases they may be of a very loose spongy formation. An examination of these cells in longitudinal section shows that these tracheal inclusions rarely, if ever, completely occlude the vessel. They extend only a short distance, usually not more than double the diameter of the cell. In unstained sections the masses are brown. They have been observed in the xylem of the main stem, petioles, stolons, and tubers (pl. 5, *B*). Such inclusions have not been

seen in the xylem of the secondary shoots and roots, but this may be due to insufficient search in these organs.

The rusty spots which are commonly associated with the basal region of the stem pith are not as prominent microscopically as they are macroscopically. In the early stages single cells are found scattered throughout the parenchyma, the walls of which take a deep safranin rather than the normal orange stain. In later stages a number of such cells may be seen arranged in an irregular group which contains one or more cells with granular or globular masses similar to those found in the necrotic phloem and showing the same stain reactions. These cells eventually collapse to form a tangled mass (pl. 4, *A*). Necrosis of the internal parenchyma is also present in the tuber but is not evident from macroscopic examination. In the cortical region of the stem, stolons, tuber, and root the effect is like that described for the internal parenchyma of the stem.

One of the most striking effects of the disease is found in the storage cells of the tuber which are normally filled with large starch grains (pl. 6, *B*, *a*). Prior to the first appearance of necrosis the starch grains migrate to the nucleus and completely surround it. Here they undergo dissolution. During this process the starch grains gradually decrease in size, lose their oval shape, and tend to become spherical. The hilum, when present, appears to become more prominent because it remains unchanged while the starch grains decrease in size. Later the hilum disappears and all that can be seen of the outline of the starch grains are the small empty spheres which remain closely surrounding the nucleus (pl. 6, *A*, *B*, *b*, *D*, *E*). Finally these fuse together and form a stringy, often granular mass.

The nucleus shows no pronounced change in its staining reaction or appearance prior to the final dissolution of the starch spheres. At this time the contents of the nucleus lose their affinity for the gentian violet and stain dark with safranin. In general, its appearance is the same as that described for the nuclei in the palisade cells during the disorganization of the chloroplasts. In some cases the shape of the nuclei of these storage cells becomes irregular. This phenomenon of starch dissolution, followed by necrosis, continues progressively in all storage cells of the plant as long as the stem remains alive.

SUMMARY

A potato disease, the origin and etiology of which are unknown, has been under observation since 1931 in West Virginia. This disease has become a limiting factor in potato production in some areas.

The first external symptoms are characterized by a dwarfing, paling, and upward folding of the terminal leaflets. Within 7 to 10 days the vines wilt and die. The vascular region of the stems, tubers, stolons, roots, and numerous regions in the pith of the stem turn brown. A discontinuous dendritic necrosis of the stem end of the tuber is one of the principal characteristics of this disease.

The anatomical features of the young terminal leaflets consist of a lateral crowding together of the palisade and mesophyll cells, failure to reach normal size, and the elimination of the intercellular spaces. These changes are accompanied by a depletion of the chloroplasts and the loss of their starch grains and vacuoles. Finally, the chloroplast

membranes disintegrate and diffuse throughout the cell. The nuclei retain their normal shape but stain heavily with safranin.

An extensive necrosis exists in the phloem and adjacent parenchyma, and to a lesser extent in the xylem and fundamental tissue. A granular deposit occurs in the necrotic areas which sometimes show lysigenous cavities. The vessels are sometimes filled with a granular material differing in staining reactions from that in the phloem and parenchyma.

Before necrosis takes place the starch grains migrate toward the nuclei, become spherical, undergo a gradual dissolution, eventually leaving behind hyaline spheres which fuse together to form a thread-like mass. The nuclei of the storage cells generally retain their normal shape and take a darker stain.

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A SIMPLIFIED METHOD OF CONSTRUCTING MERCHANTABLE BOARD-FOOT VOLUME TABLES[1]

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INTRODUCTION

Several methods of preparing tables to show the merchantable contents of standing trees in board feet, or in some other unit of measure, are in customary use. All these methods have two disadvantages in common: They require large numbers of detailed field measurements; and the computation of tables by any of them is a rather lengthy and difficult process, particularly for anyone not specially trained in mensuration.

In addition to the difficulties of preparation, the tables themselves have certain shortcomings. Two basic types of merchantable board-foot volume tables are most often used. The first type relates volume to diameter at breast height and at total height of the tree; the second is based on diameter at breast height and "merchantable height", or the distance from the ground to a point on the trunk of the tree with a given fixed diameter. In the first type of table, although the volume is based on total height, the actual volume given is that of the trunk up to a point of fixed top diameter. In both types, therefore, the point of merchantability is actually determined solely by minimum diameter. This is a disadvantage, because in actual utilization merchantability is much more often limited by the presence of large limbs or deformities of the bole (particularly in hardwoods).

The second type of table has the additional disadvantage of being difficult to use correctly in the field, because of the necessity of estimating the point on the trunk, usually quite high above ground, where the fixed top diameter occurs.

A method of preparing volume tables which takes into account the fact that the position of large branches and irregularities of the bole more often determine the actual upper limit of merchantability than does an assumed minimum diameter, would seem to have practical advantages. Such a method is described in this paper.

FACTORS INFLUENCING VOLUME DIFFERENCES AMONG SPECIES

The common practice in forest mensuration is to use a different volume table for each species and sometimes even for the same species in different localities. It may be assumed, therefore, that the correlation between the dependent variable, volume, and the two independent variables, diameter and height, is definitely affected by species, for if all trees had the same form, the same bark thickness, and the same degree of butt swell, volume would vary with height and diam-

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eter in exactly the same way for all species. Within species these factors are relatively constant, and in some cases a number of species are so similar as to permit of grouping.

Numerical expressions for these several factors were worked out in this study for 14 species from three forest regions. By using these expressions it was possible actually to measure the difference between species. A single basic correlation of volume with diameter and height was determined. This can be modified by the use of these numerical expressions so that it will fit any species or particular sample of a species.

BARK THICKNESS

The first factor which causes a difference between species is bark thickness. The diameter measurement upon which the volume estimate is based is made outside the bark, while the volume is measured inside the bark. Thus it is clearly evident that, if the bark of one species averages 10 percent of the diameter and the bark of a second species is 7 percent of the diameter, the correlation between volume and diameter for each of the species will be different. In order to eliminate this factor as a source of difference between species, the diameter breast high [2] used in the basic correlation of volume with diameter and height is measured, in this method, inside the bark instead of outside.

BUTT SWELL

Another factor which is fairly constant within a species but different between species is butt swell, which affects the measurement of diameter at breast height. Trees with a large degree of butt swell have less volume for a given diameter than do trees with only a slight degree. A satisfactory numerical measure of butt swell was found to be afforded by the ratio of the diameter at 18.3 feet above the ground (top of first 16.3-foot log above a 2-foot stump) to the diameter at breast height, both measurements being made inside the bark.[3] This factor is denoted as B. It is relatively constant within a species, at least within rather broad geographical limits. Table 1 shows the average value of B and its standard deviation for a number of species.

TABLE 1.—*Average value and standard deviation of B (butt swell factor), by species*

Species	Average value of B	Standard deviation	Trees	Species	Average value of B	Standard deviation	Trees
Hardwoods:				Conifers:			
Basswood (*Tilia glabra* Vent.)	0.83	0.054	*Number* 275	Balsam fir (*Abies balsamea* (L.) Mill.)	.81	.047	*Number* 81
Beech (*Fagus grandifolia* Ehrh.)	.86	.043	339	Hemlock (*Tsuga canadensis* (L.) Carr.)	.85	.041	240
Elm (*Ulmus americana* L.)	.84	.048	140	Jack pine (*Pinus banksiana* Lamb.)	.86	.042	57
Red gum (*Liquidambar styraciflua* L.)	.81	.046	119	Loblolly pine (*P. taeda* L.)	.90	.046	106
Red maple (*Acer rubrum* L.)	.84	.037	131	Norway pine (*P. resinosa* Ait.)	.90	.073	186
Sugar maple (*A. saccharum* Marsh.)	.86	.046	553	Shortleaf pine (*P. echinata* Mill.)	.91	.037	59
Yellow birch (*Betula lutea* Michx. f.)	.84	.040	296	White pine (*P. strobus* L.)	.88	.034	80

[2] 4.5 feet above ground level.

[3] This factor was suggested by a form-class measurement developed by J. W. Girard, but differs in that in this case both measurements are taken inside the bark; in Girard's form class, the measurement at top of first log is inside bark, but diameter breast high is measured outside bark.

FORM

Form or taper in the upper part of the bole is a third factor which causes variation in volume between species. Several numerical measures of the form of trees have been devised, the most common of which is the ratio of the diameter at half the height of the tree to the diameter at breast height. Several minor modifications of this form quotient have also been used. These expressions of form all have the same weakness—the basic measurement is diameter breast high, and since diameter at breast height is often affected by butt swell, the form quotient is likewise influenced by the degree of butt swell whereas it should vary solely with taper. In this analysis a factor was found which avoids this source of variation. This is the ratio of the diameter inside bark at 34.6 feet [4] above ground to the diameter inside bark at 18.3 feet above ground, hereafter denoted as D. Table 2 shows the average value and standard deviation of D for a number of species.

TABLE 2.—*Average value and standard deviation of D (factor of form), by species*

Species	Average value of D	Standard deviation	Trees	Species	Average value of D	Standard deviation	Trees
Hardwoods:			*Number*	Conifers:			*Number*
Basswood	0.89	0.040	275	Hemlock	0.86	0.072	221
Beech	.89	.038	219	Jack pine	.82	.057	57
Red gum	.87	.047	119	Loblolly pine	.87	.041	106
Sugar maple	.89	.041	377	Norway pine	.89	.074	187
Yellow birch	.87	.041	157	Shortleaf pine	.85	.048	59
				White pine	.91	.038	80

By controlling these three factors, bark thickness, butt swell (B), and form (D), it was possible to determine a correlation between volume and diameter which is constant for all species.

THE BASIC CURVES OF VOLUME

Stem measurements of approximately 2,000 trees were used in developing the set of curves which is the basis for the system of volume-table construction presented. These trees were first classified by log lengths. The trees in each log-length class were then sorted into D classes and subsorted into B classes. Thus, in the final grouping, log length, D, and B were all constant within a classification interval. Merchantable volume in board feet was then plotted over diameter breast high inside bark with a separate curve plotted for each of the final groups. In effect, these curves represent the correlation of volume with diameter breast high inside bark, with the other factors (butt swell and form) held constant—in statistical terms, the net regression of volume with diameter breast high inside bark.

Within each log class, the curves were parallel and in logical order one above the other, indicating that there was no joint relationship between volume and the three variables, diameter breast high, B, and D. Therefore, a single curve was drawn for each log class, parallel to the average trend of the series for that log class and through the ordinates which represented the average values of B and D. The final set of curves is illustrated in figures 1 to 5.

[4] Equal to two 16-foot logs plus trimming allowance and a 2-foot stump.

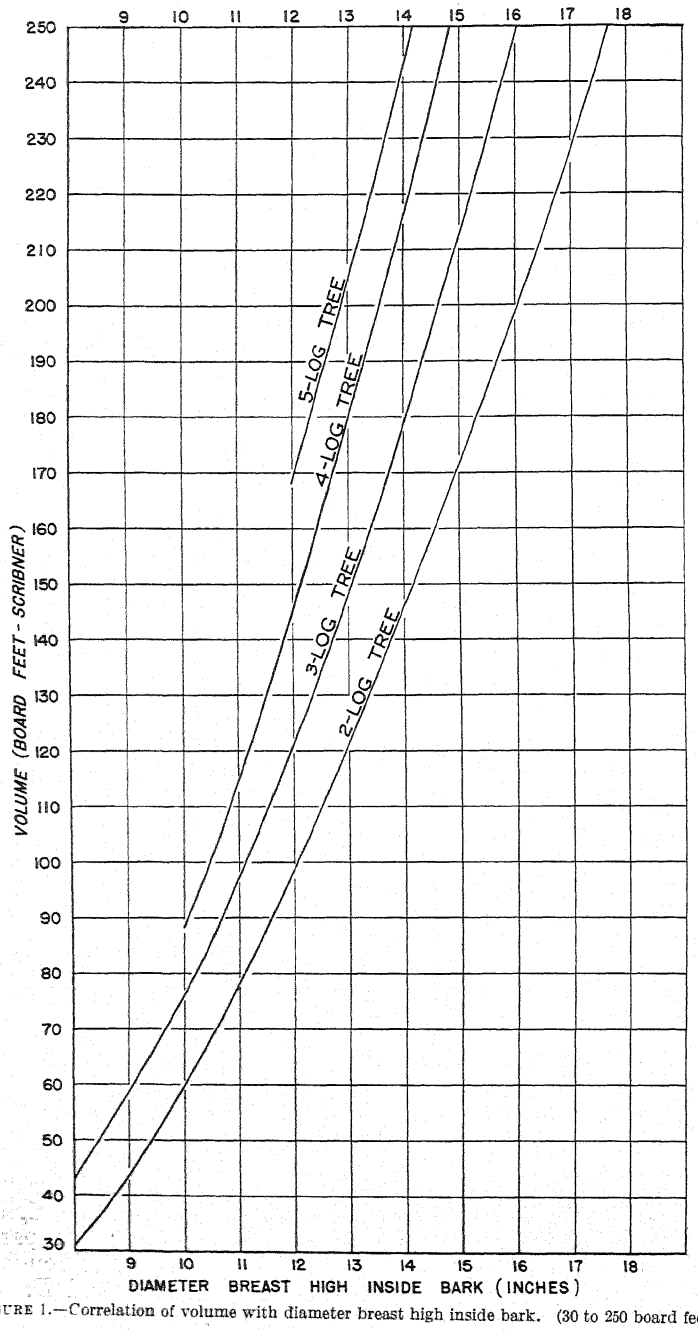


FIGURE 1.—Correlation of volume with diameter breast high inside bark. (30 to 250 board feet.)

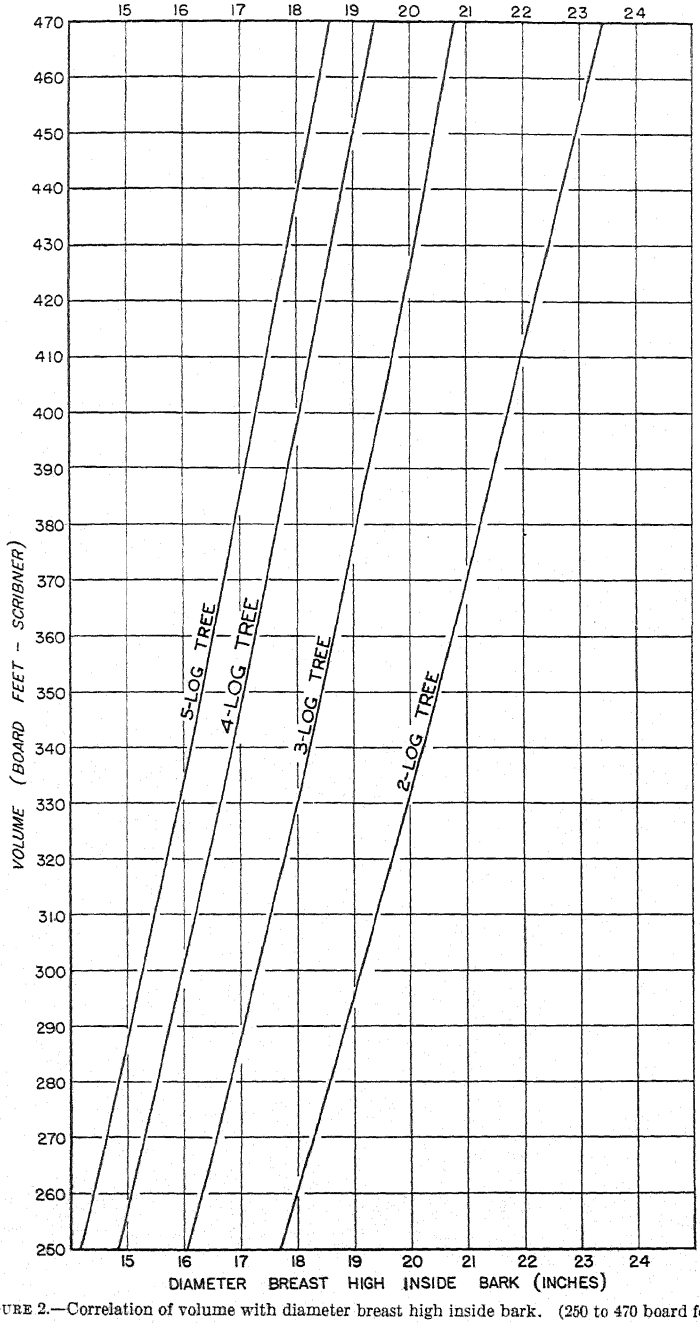


FIGURE 2.—Correlation of volume with diameter breast high inside bark. (250 to 470 board feet.)

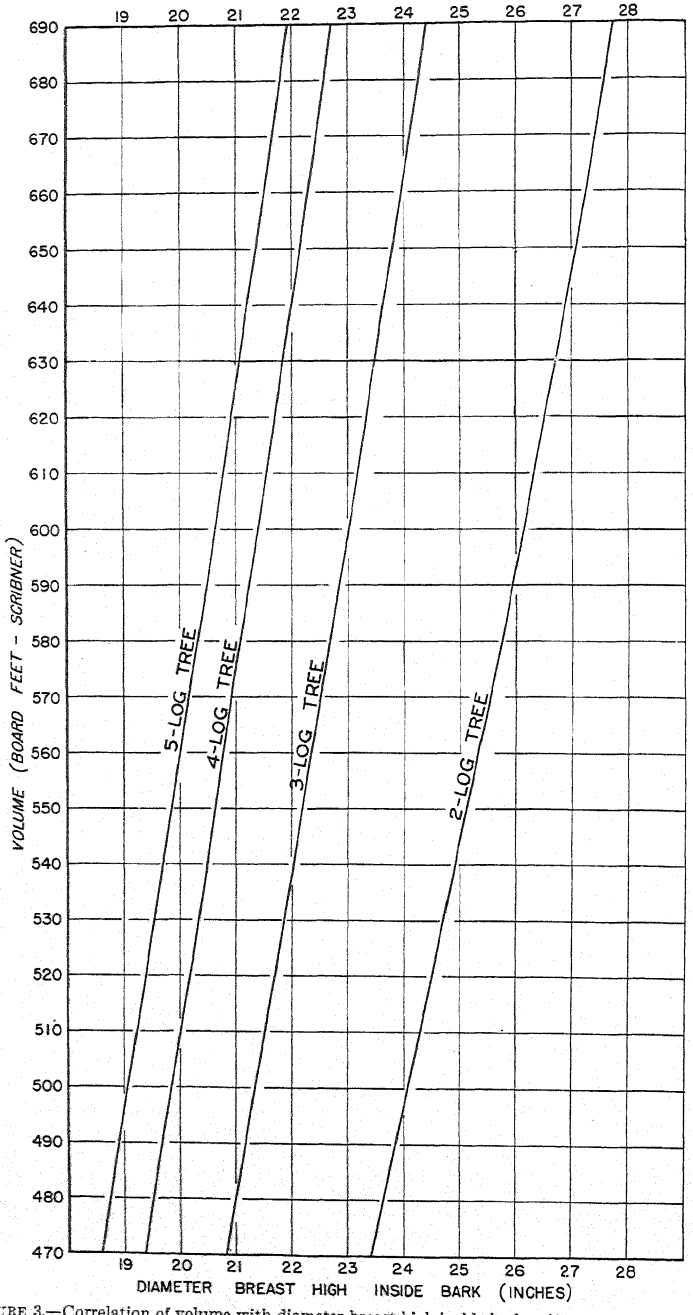


FIGURE 3.—Correlation of volume with diameter breast high inside bark. (470 to 690 board feet.)

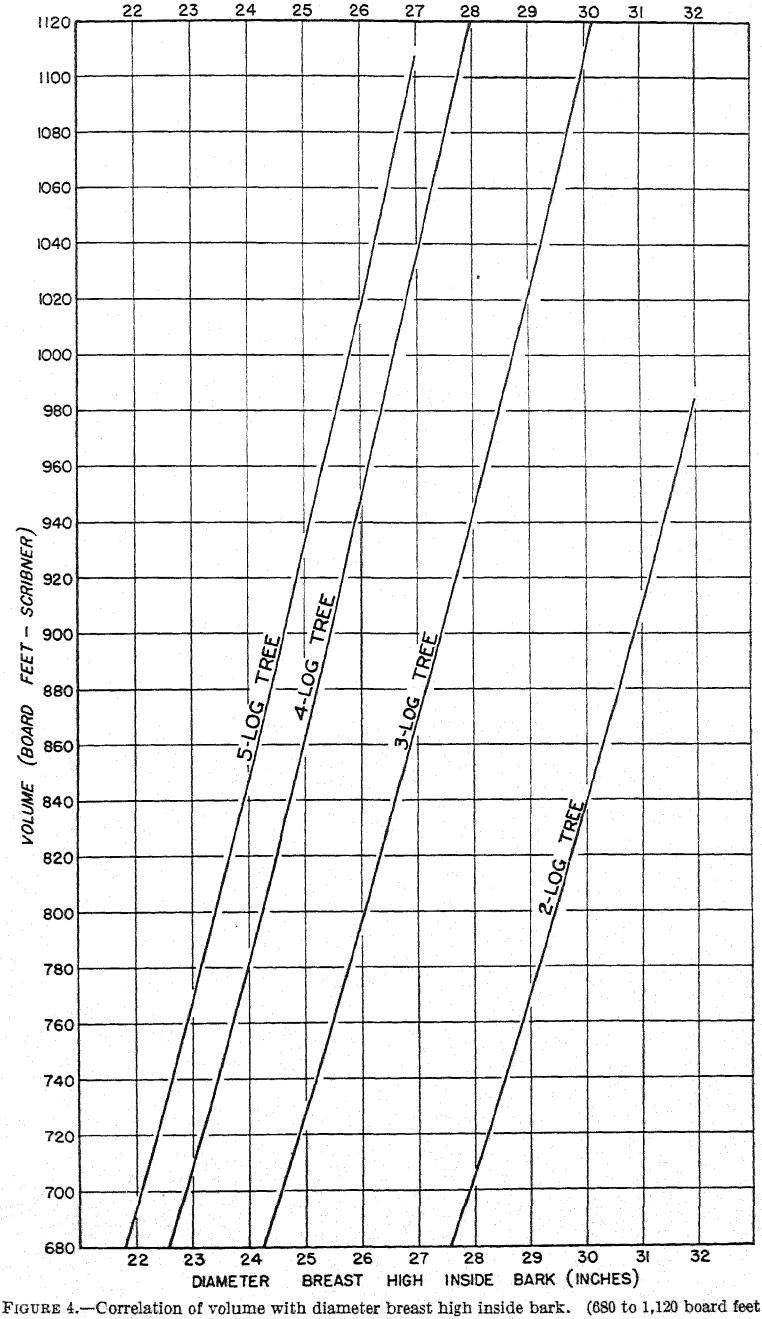


FIGURE 4.—Correlation of volume with diameter breast high inside bark. (680 to 1,120 board feet.)

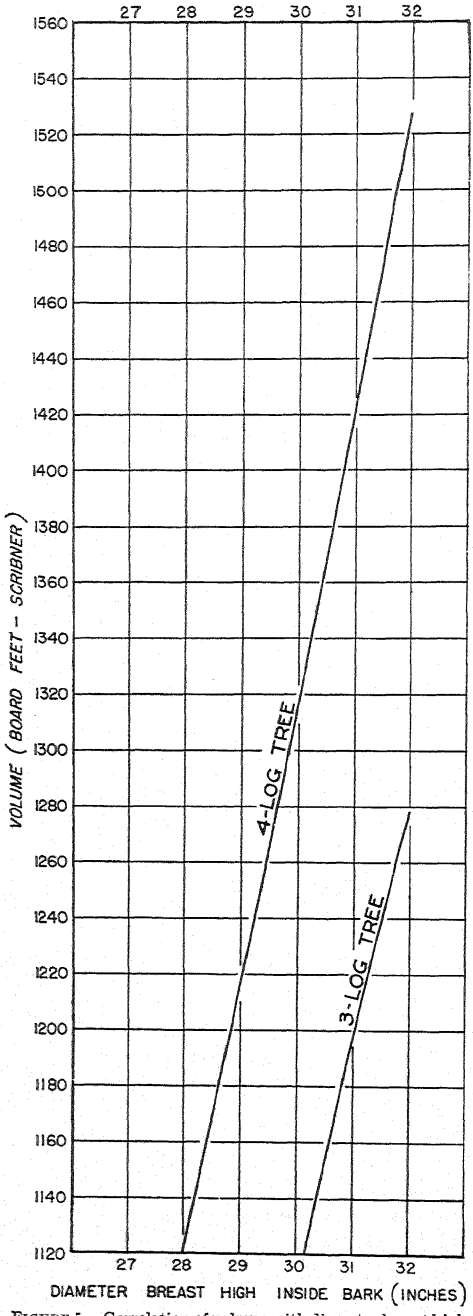


FIGURE 5.—Correlation of volume with diameter breast high inside bark. (1,120 to 1,560 board feet.)

Next, the volume of each sample tree was estimated from the curves, and this estimated volume was divided into the actual volume of the tree (determined from individual stem measurements). The quotients thus obtained were denoted as R. For trees with less butt swell and with better form than average, the R's are greater than 1.0; for trees of poor form and large degree of butt swell, the R's are less than 1.0.

These R's were then correlated with D and B. The trees were sorted into B classes, and a curve of R over D was drawn for each B class. Here again, the curves were parallel and in logical order, indicating the absence of joint relationships. A single curve (fig. 6) was, therefore, drawn to represent the net trend of R with D. The R was then estimated from this curve for each sample tree, and the differences were computed between these estimated R's and the actual R's. These residuals (termed r) were then plotted over B, and a curve fitted (fig. 7). From this curve a correction was read for each sample tree and applied to the value of R as estimated from the curve of R over D. These estimated and corrected R's were then compared with the actual R's.

The differences between the corrected R's and the actual R's were then sorted by log classes and subsorted by diameter classes. Next

the differences were converted into board feet and plotted against the original set of basic curves of volume over diameter breast high inside bark. These curves were then redrawn wherever it appeared necessary. New volumes were estimated from these curves, new R's computed, and these R's again correlated with the D and B factors just as in the first approximation. This process was repeated several times until no further adjustments in the fit of any of the curves were necessary. All of the curves were fitted freehand.

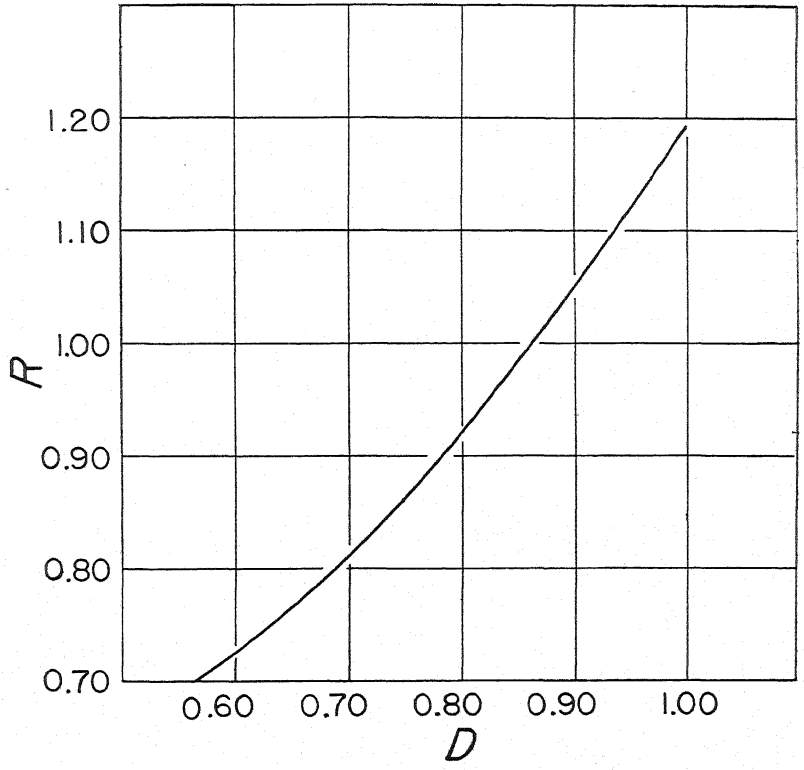


FIGURE 6.—Correlation of R with D.

When all the curves had been finally fitted, the differences between the actual R's and the estimated R's were sorted by species. If the factors used in constructing volume tables by this method are really effective in measuring the differences between species, the final average residual of one species should not be significantly different from that of another species, and they should all be close to zero. Table 3 shows the average residual of R for each of the species used in developing the method.

The average residuals of R shown in table 3 are numerically equivalent to the average deviations of estimated volume from actual volume expressed as a percent of the estimated volume.

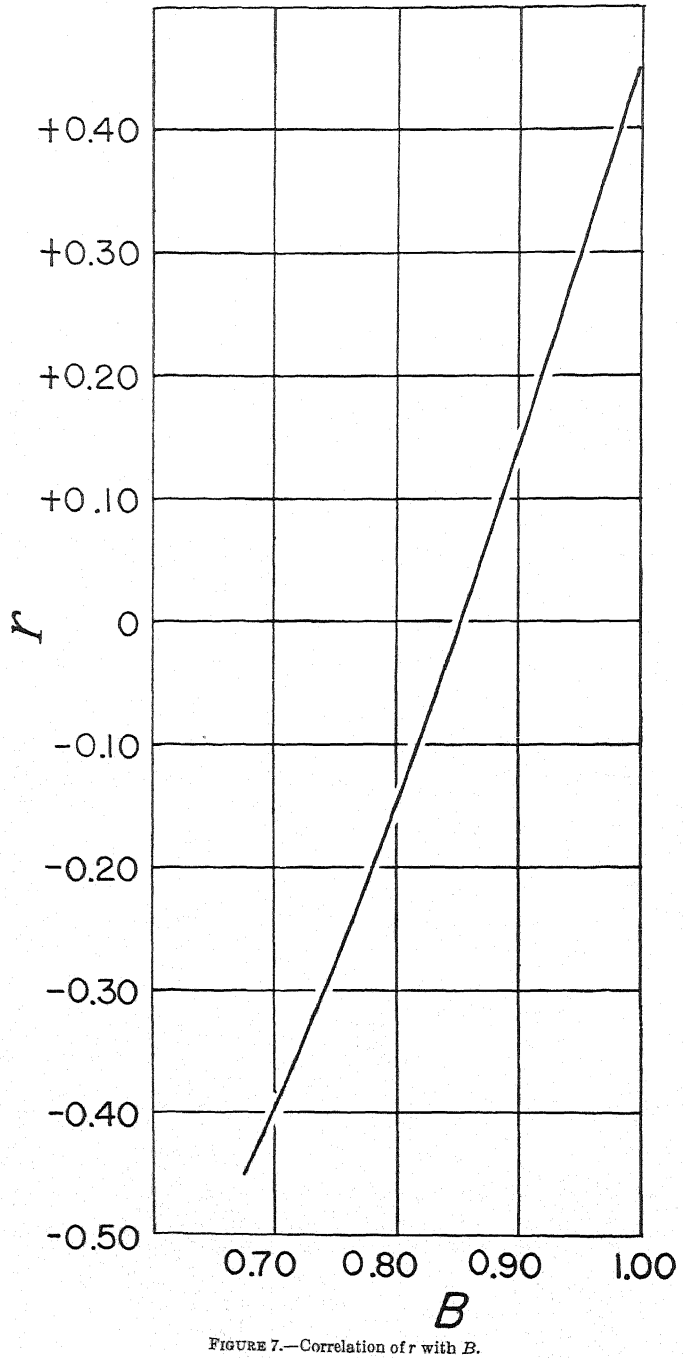


FIGURE 7.—Correlation of r with B.

TABLE 3.—*Average residuals by species*

Species	Trees	Average residual	Species	Trees	Average residual
	Number	*Percent*		*Number*	*Percent*
Sugar maple	166	+0.31	Jack pine	57	−1.12
Yellow birch	137	−.31	Norway pine (second growth)	117	−.50
Basswood	275	+.70	Norway pine (old growth)	183	+1.80
Hemlock	239	−.70			

Fisher's analysis of variance test[5] was used to determine the significance of the differences between the observed residuals. Although the test showed that the differences are significant, they are actually so small and so slightly removed from zero that the fact of statistical significance is of little practical importance. It should be noted (table 3) that the difference between the two groups of Norway pine is nearly as great as the difference between the most extreme cases (Norway pine old growth and jack pine). The residuals are chiefly the result of variations in volume associated with taper in the portion of the tree above two logs, over which portion the method affords no direct control.

As a test the volumes of several groups of trees which had not been used in developing the method were estimated and the residuals listed in table 4 were obtained.

TABLE 4.—*Average residuals of species not used in developing the method*

Species	Trees	Average residual	Species	Trees	Average residual
	Number	*Percent*		*Number*	*Percent*
Shortleaf and loblolly pines	165	−0.41	Sugar maple	210	−.20
Mixed southern hardwoods	79	−.95	White pine	80	+.69
Red gum	118	−.84			

The index of correlation is another measure of the effectiveness of the method. In order to test the theory of the method rather than its accuracy under field conditions, the volume of each sample tree was estimated, using its individual D and B factors, a method which is not practicable under field conditions. By working in this manner, a correlation index of 0.9765 was obtained. Thus it was demonstrated that a very large proportion (95 percent) of the variance in volume is accounted for by the variables taken into consideration.

APPLICATION OF THE METHOD

COLLECTION OF DATA

In collecting sample tree measurements to be used in constructing volume tables by this method, the objective should be to sample as well as possible the area to which the table is to be applied. The number of trees to be measured depends on the accuracy desired and the uniformity of the area. On the basis of this study it appears that 100 trees will generally give average values of D and B with standard errors of less than 0.005. For use in restricted areas, where greater

[5] FISHER, R. A. STATISTICAL METHODS FOR RESEARCH WORKERS. Ed. 4, 307 pp., illus. Edinburgh and London. 1932.

constancy of form and butt swell can be expected, a smaller number of trees will suffice.

Only four measurements are needed from each sample tree. These are (1) diameter breast high outside bark, (2) diameter breast high inside bark, (3) inside-bark diameter at 18.3 feet above the ground, and (4) inside-bark diameter at 34.6 feet above the ground. Only trees with a reasonably normal form should be measured; trees with pronounced irregularities at the points of measurement should be avoided. Even though such trees do occur in the area to which the volume table is to be applied, their inclusion among the sample trees only adds inaccuracy to the estimate of the normal trees.

COMPUTATION OF FORM AND BUTT SWELL FACTORS

After the measurements have been collected, the next step is to compute the values of the two factors, B and D, for each tree, using the following formulas:

$$B=\frac{\text{Diameter inside bark at 18.3 feet}}{\text{Diameter inside bark at breast height}}$$

$$D=\frac{\text{Diameter inside bark at 34.6 feet}}{\text{Diameter inside bark at 18.3 feet}}$$

After these two factors have been computed for each sample tree, either of two methods may be followed, depending upon the amount of data on hand and the accuracy desired. The first and simplest method is to determine the arithmetic average of each of the two factors by summing the individual values and dividing by the number of observations. The second method involves the correlation of B and D with diameter and height. This scheme is merely a modification of the first method and follows it analogously.

The following numerical example illustrates the computation. Measurements were first taken on 296 yellow birch trees in the Upper Peninsula of Michigan. From these data the average D was found to be 0.87 and the average B 0.84. The next step is to determine R from the curve shown in figure 6, or from the values in table 5.

TABLE 5.—*Values of R and D, as read from figure 6*

D	Value of R for hundredths indicated									
	0.00	0.01	0.02	0.03	0.04	0.05	0.06	0.07	0.08	0.09
0.60	0.73	0.73	0.74	0.75	0.76	0.77	0.77	0.78	0.79	0.80
.70	.81	.82	.83	.84	.85	.86	.88	.89	.90	.91
.80	.92	.93	.95	.96	.97	.98	1.00	1.01	1.02	1.04
.90	1.05	1.06	1.08	1.09	1.11	1.12	1.14	1.15	1.16	1.18

From this table or the curve of figure 6 the R value which corresponds to a D of 0.87 is found to be 1.01.

The next step is to determine the correction factor to be applied to R; this factor is known as r and is found in figure 7 or table 6 by looking up the r which corresponds to the average value of B (0.84).

TABLE 6.—*Values of r and B as read from figure 7*

B	Values of r for hundredths indicated									
	0.00	0.01	0.02	0.03	0.04	0.05	0.06	0.07	0.08	0.09
0.60								−0.46	−0.44	−0.42
.70	−0.40	−0.38	−0.35	−0.33	−0.30	−0.28	−0.26	−.23	−.20	−.18
.80	−.15	−.12	−.10	−.07	−.04	−.01	+.02	+.05	+.08	+.11
.90	+.14	+.17	+.20	+.23	+.26	+.29	+.32	+.35	+.38	+.42

In this case r equals −0.04. This value is then applied (added or subtracted according to the sign given in the table) to the value of R already found, and the result, in this case 0.97 (1.01−0.04=0.97), is used to adjust the basic table of volume over diameter inside bark. These volumes are presented in figures 1 to 5 and also in tabular form in table 7.

TABLE 7.—*Volume in board feet, Scribner decimal C rule by diameter breast high, inside bark, and merchantable length in 16.3-foot logs*

Diameter breast inside bark (inches)	Volume by merchantable length in number of 16.3-foot logs				Diameter breast inside bark (inches)	Volume by merchantable length in number of 16.3-foot logs			
	2	3	4	5		2	3	4	5
	Board feet	*Board feet*	*Board feet*	*Board feet*		*Board feet*	*Board feet*	*Board feet*	*Board feet*
8	31	43	-------	-------	21	370	480	574	624
9	44	59	-------	-------	22	412	538	640	694
10	59	76	88	-------	23	453	598	708	767
11	78	97	114	-------	24	497	663	781	845
12	98	121	145	168	25	544	728	861	930
13	121	148	179	204	26	593	795	946	1,016
14	146	178	216	243	27	645	868	1,034	1,107
15	171	213	256	287	28	705	942	1,121	-------
16	198	249	301	334	29	768	1,021	1,217	-------
17	228	288	346	385	30	837	1,106	1,317	-------
18	260	330	398	438	31	908	1,197	1,421	-------
19	295	377	450	496	32	984	1,278	1,527	-------
20	332	426	509	559					

All that remains is to multiply the curved or tabular volumes by 0.97 and then convert to an outside-bark basis.[6]

PROCEDURE FOR ONE-LOG TREES

It will be noticed that there is no volume curve for one-log trees. Since the merchantable length of these does not extend to 34.6 feet it is not possible to compute the factor D for one-log trees. However, the product of the average value of the factor B and the inside-bark diameter at breast height of any tree is equal to the diameter inside bark at the top of the first 16.3-foot log of that tree, which, in the case of one-log trees, makes up the entire volume. Therefore, it is necessary only to look up the volume of a log of this diameter in table 8 to determine the volume of the tree. Using, for example, the average B found for yellow birch trees, 0.84, to find the volume of a tree

[6] In actual practice, time can be saved by reading from a curve of inside-bark diameter at breast height over outside-bark diameter at breast height the inside-bark diameters which correspond to whole inch values of outside-bark diameters, and then reading the volumes which correspond to these fractional values of inside-bark diameter from the curves.

with a diameter breast high inside bark of 14.8 inches (corresponding to an outside-bark diameter at breast height of 16 inches) it is necessary to multiply the diameter breast high by the factor (14.8×0.84). The result, 12.4 inches, is the diameter inside bark of the top of the first log. The volume of this log is found, in table 8, to be 93 board feet, which is then assigned as the volume of one-log trees with an outside-bark diameter breast high of 16 inches. This procedure is followed for all diameter classes. In order to avoid interpolation or curving, it is advisable to determine the inside-bark diameters at breast height which correspond to whole inch values of diameter breast high outside bark before computing the volumes.

TABLE 8.—*Scale of 16-foot logs read to nearest board foot from Scribner Decimal C rule computed by formula* $V=0.79D^2-2D-4$

Diameter inside bark (inches)	Volume for tenths of inch indicated									
	0.0	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9
	Bd. ft.	*Bd. ft.*	*Bd. ft.*	*Bd. ft.*	*Bd. ft.*	*Bd. ft.*	*Bd. ft.*	*Bd. ft.*	*Bd. ft.*	*Bd. ft.*
6	12	12	13	14	15	16	17	18	19	20
7	21	22	23	24	24	25	26	27	28	29
8	30	31	32	33	34	36	37	38	39	41
9	42	43	45	46	47	48	50	51	52	54
10	55	56	58	60	61	63	64	66	67	69
11	70	72	74	75	77	78	80	81	83	84
12	86	88	90	91	93	95	97	99	101	102
13	104	106	108	110	111	113	115	117	119	121
14	123	125	127	129	131	133	135	137	140	142
15	144	146	148	150	153	155	157	159	161	164
16	166	168	171	173	175	177	180	182	185	187
17	189	191	194	196	199	202	204	207	210	213
18	216	218	221	224	227	229	231	234	237	240
19	243	245	248	251	254	257	260	263	266	269
20	272	275	278	281	284	287	290	293	296	299
21	302	305	308	311	314	317	320	323	327	330
22	334	337	340	344	348	351	354	358	361	365
23	368	372	375	379	382	386	390	394	397	400
24	403	407	410	414	417	422	425	428	431	435
25	440	443	447	450	455	458	462	466	470	474
26	478	482	486	490	494	497	502	506	510	514
27	518	522	526	530	534	537	542	546	550	554
28	559									
29	602									

MODIFIED PROCEDURE

If very exact results are desired and there is sufficient material at hand, the values of D and B can be correlated with diameter breast high alone or with diameter breast high and height. In many cases the correlation is so slight that it can be ignored and the simple averages of D and B used. When a fair degree of correlation is found, however, the volume table can be improved by estimating D and B for each diameter class separately and determining the corresponding corrected R's. Once the curves of D and B have been established, this procedure is equally simple and direct.

A number of other modifications are possible and will suggest themselves to anyone working with the method.

APPLICATION OF THE TABLES IN THE FIELD

Much of the dissatisfaction with the older types of tables has its origin in the application of the tables in the field. When properly used they give excellent results, but it is necessary to follow exactly the specifications which were set up when the tables were constructed.

Tables of the type described here can be used with much greater flexibility and with little chance of mistakes in application. The tables present the volume in board feet (according to the Scribner decimal C rule computed by the formula $V=0.79D^2-2D-4$), above a 2-foot stump and up to whatever height the tree is merchantable regardless of diameter at that point, provided it is over 6 inches. The merchantable height should be estimated to the point where actual utilization will cease, which in most cases will be determined by the presence of large limbs or bole deformities. So long as the merchantable length is not carried up into the top where the main stem becomes rough and irregular, the table will give highly accurate results. Field checks have shown that the volumes given agree very closely with the actual volume removed in logging operations.

If there are species which have extremely low values of both D and B (below 0.65–0.78) the volumes as given by this method will be slightly low. Such low values, however, were not found in any of the species used in this study, and are unusual.

SUMMARY

A new and simple method of constructing board-foot volume tables is presented. This method takes into account the fact that the upper limit of merchantability is more often fixed by the position of large limbs or deformities than by minimum diameter. A set of basic curves of volume over diameter at breast height, inside bark, serves as the foundation. These curves are modified by the use of two numerical measures of form and butt swell so that they will fit any species coming within the size range of the material presented.

The construction of tables by this method presents several advantages not found in other methods. The collection of data is simplified because only a relatively few trees must be measured and only four measurements are required from each tree. In the computation of the field data only two simple ratios need to be worked out for each tree and an average of each computed. It is not necessary to determine the actual volume of the trees. There is the added advantage that only one result is possible—there is no chance for variation between tables made by several workers from the same data. Thus, no highly specialized knowledge is necessary for the construction of tables by this method.

Accuracy of the volumes computed from this type of table has been demonstrated by actual measurement of logs.

RHYNCHOSPORIUM SCALD OF BARLEY, RYE, AND OTHER GRASSES[1]

By RALPH M. CALDWELL

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INTRODUCTION

Scald of barley, rye, and other grasses, caused by *Rhynchosporium* spp. is a common foliage disease in many parts of the world. In certain regions of North America it has been one of the principal limiting factors of barley production. Little study has been given this disease by pathologists in the United States and only slightly more in Europe. The present studies, initiated in Wisconsin in 1926, comprise a general consideration of the taxonomy, physiology, and host specialization of the causal fungus and of the host-parasite relationships, and seasonal development of the disease. The findings relative to physiologic specialization and pathological histology stand in marked contrast to those of Bartels (*1*) [3] in Germany and Brooks (*2*) in England. Two preliminary reports have been published on this work (*3*, *4*).

THE DISEASE

COMMON NAME

Several common names have been applied to the disease referred to as "scald" in this paper. These include "leaf blight", "leaf spot", "leaf blotch", and "scald." With the exception of the latter, each of these has been used to designate another cereal disease and is avoided here to prevent confusion. The term "scald", besides being distinctive among cereal disease names, has in its favor the facts that it is accurately descriptive of the disease in its most aggressive form and that recently it has been frequently used.

HISTORY, DISTRIBUTION, AND ECONOMIC IMPORTANCE

Oudemans (*17*) first recorded the discovery of the scald organism in June 1897, having found it on rye (*Secale cereale*) in the Netherlands. He reported it under the name *Marsonia secalis* n. sp. Frank (*11*), in October 1897, gave the first adequate description of the disease, reporting it on barley and rye in Germany. He recognized it to be of considerable economic importance on barley, particularly when the plants were attacked in advance of the heading stage. Seedling plants of barley were reported to be killed by severe attack. Frank's examination of herbarium specimens revealed the presence of the disease in Germany in 1894, which is its earliest known occurrence.

[1] Received for publication Apr. 13, 1937. Issued August 1937. Cooperative investigations by the Division of Cereal Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture, and the Departments of Plant Pathology and Botany of the University of Wisconsin, and the Department of Botany, Purdue Agricultural Experiment Station.

[2] The writer gratefully acknowledges the assistance of Drs. J. G. Dickson and E. M. Gilbert of the University of Wisconsin during the progress of these studies.

[3] Reference is made by number (italic) to Literature Cited, p. 198.

Frank reported inoculation experiments by E. Heinsen proving the relationship of the causal fungus. Heinsen (*12*), in 1901, published a full account of his experiments with scald, referred to by Frank. His inoculations showed barley and rye to be susceptible, wheat slightly susceptible, and oats immune.

Since these early papers scald has received little attention other than in records of occurrence and a report of resistance in barley by Johnson and Mackie (*13*), until the recent publications of Brooks (*2*), Bartels (*1*), and Mackie (*16*). The disease was first mentioned in American literature in 1917,[4] when it was reported to have been prevalent in 1915 in the United States. It is now known to occur widely in northern Europe and in the United States, and in Canada, New South Wales, Tunis, Argentina, and Peru. Reports of severe losses from scald have come only from the United States and Germany. The disease occurs very frequently in severe form on the winter barley crop of the Pacific Coast States and Idaho. The most destructive epidemics have been reported in the interior valleys of California where a positive correlation was noted between scald resistance and yield (*6*). Barley yields in this region have been estimated to be reduced as much as 20 to 30 percent (*5*). In the same State in 1925,[5] the disease was reported as "killing the leaves, shrivelling the kernels, and weakening the plants to such an extent that a reduction of yield of probably 25 percent occurred." Wiebe [6] makes the following report of barley scald in California, based upon comparative yields of susceptible and resistant varieties during epidemic and nonepidemic years: "In 1935 barley scald was a serious disease in California. Yields of susceptible varieties were reduced from 10 to 15 percent." Less frequently scald may become prevalent in other sections of the United States but has not been considered of major economic significance. In spring barley plots at Madison, Wis., in 1928, 1929, and 1930, the disease became abundant during the booting and heading stages of growth. The foliage was almost entirely killed, yet the plants matured and produced apparently normal heads and grain. Although yield reduction must have resulted, no measure of the loss could be made. In Europe, scald has been reported as severe on the seedling and mature plants by Frank (*11*) and Heinsen (*12*). Brooks (*2*) states that susceptible varieties in England, although suffering loss of almost all the lower leaves, recovered as the plants matured, and showed no appreciable effect of the disease at harvest time.

PLANTS ATTACKED

In addition to very commonly attacking barley (*Hordeum vulgare* L. and *H. distichum* L.) and rye (*Secale cereale* L.), scald has been reported twice on wheat (*Triticum aestivum* L., *T. vulgare* Vill.), once in Germany (*12*), and once in Washington State in the United States.[7] It was also once reported on oats (*Avena sativa* L.) in Denmark (*14*).

A number of grasses belonging to seven genera are also reported to be attacked. The literature to date lists the following occurrences of this disease on grasses, namely, *Agropyron repens* (L.) Beauv. in

[4] UNITED STATES BUREAU OF PLANT INDUSTRY. DISEASE CAUSED BY RYHNCHOSPORIUM GRAMINICOLA. U. S. Bur. Plant Indus. Plant Disease Bull. 1: 9. 1917. [Mimeographed.]

[5] HASKELL, R. J. SCALD CAUSED BY RHYNCHOSPORIUM SECALIS (OUD.) DAVIS. U. S. Bur. Plant Indus. Plant Disease Reptr. Sup. 48: 341. 1926. [Mimeographed.]

[6] Personal correspondence with G. A. Wiebe, Division of Cereal Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture.

[7] JOHNSON, A. G., and HASKELL, R. J. RHYNCHOSPORIUM GRAMINICOLA . . . U. S. Bur. Plant Indus. Plant Disease Bull. Sup. 8: 37. 1920. [Mimeographed.]

Wisconsin (*3*, *8*), Oregon;[8] Denmark (*14*), and Germany (*1*); *A. dasystachyum* (Hook.) Scribn. in Oregon;[8] *Bromus inermis* Leyss. in Wisconsin (*3*, *9*); *B. mollis* L. in England (*2*); *B. sterilis* L. in England (*2*); *Dactylis glomerata* L. in Wisconsin (*9*), England (*2*), and Oregon;[8] *Danthonia* sp. Lam. and DC. in Oregon;[9] *Elymus robustus* Scribn. and J. G. Sm. (*canadensis*) in Wisconsin (*4*); *E. glaucus* Buckley in

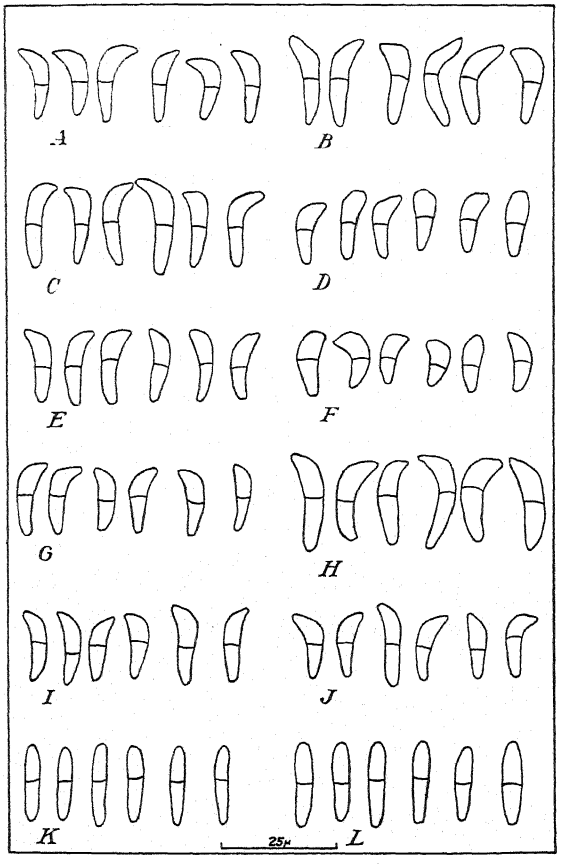


FIGURE 1.—Morphology of conidia of races of *Rhynchosporium secalis* and of *R. orthosporum*, produced on their respective hosts and in cultures isolated from these hosts. *A*, *B*, Barley race of *R. secalis* from host and culture respectively; *C*, *D*, rye race of *R. secalis* from host and culture respectively; *E*, *F*, *Agropyron repens* race of *R. secalis* from host and culture respectively; *G*, *H*, *Bromus inermis* race of *R. secalis* from host and culture respectively; *I*, *J*, *Elymus canadensis* race of *R. secalis* from host and culture respectively; *K*, *L*, *R. orthosporum* from host and culture respectively.

Oregon;[8] *Hordeum jubatum* L. in Wisconsin (*3*) and Oregon;[8] *H. murinum* L. in Germany (*1*) and Oregon;[8] *Lolium perenne* L. in Germany (*1*) and Oregon;[8] *L. multiflorum* Lam. in Oregon;[8] *Milium effusum* L. in Denmark (*14*). In addition, the writer has found the disease on *Elymus virginicus* L. in Indiana and on *Hordeum jubatum* L. in South Dakota.

Dactylis glomerata has been found by the writer in Wisconsin attacked by an undescribed species of *Rhynchosporium* differing from

[8] SPRAGUE, R. A PRELIMINARY CHECK LIST OF THE PARASITIC FUNGI ON CEREALS AND OTHER GRASSES IN OREGON. U. S. Bur. Plant Indus. Plant Disease Reptr. 19: [156]-186. 1935. [Mimeographed.]
[9] HASKELL, R. J. See footnote 5.

R. secalis in the cylindrical shape and lack of apical beak of the conidia (fig. 1, *K*, *L*). The symptoms of the disease are very similar to those caused by *R. secalis* on other hosts. An examination of the collection from which Drechsler (*9*) reported *R. secalis* on this host has shown the conidia to be those of this undescribed species. This species has also been collected by Sprague[8] in Oregon and submitted to the writer for identification. Thus, the only two reports of *Rhynchosporium* on *D. glomerata* in the United States have proved to be based on the new species rather than on *R. secalis*. The description of the new species, designated as *R. orthosporum* sp. nov., is given herein in the section on Taxonomy and Nomenclature.

A number of grass species not reported as naturally infected in the field have been infected by artificial inoculation with *Rhynchosporium secalis*. Bartels (*1*) reports the following species artificially infected with inoculum collected in Germany: *Lolium italicum*, *Poa pratensis*, *P. nemoralis*, *P. compressa*, *P. trivialis*, *Bromus arvensis*, *Agrostis stoloniter*, *Cynosurus cristatus*, *Phleum pratense*, and *Holcus lanatus*. The writer has artificially infected the following species, unknown to be naturally infected: *Agropyron tenerum*, *Bromus arenarius*, *B. lanuginosus*, *B. madritensis*, *B. villosus*, *Elymus striatus*, *Hordeum nodosum*, and *H. pusillum*.

SYMPTOMS

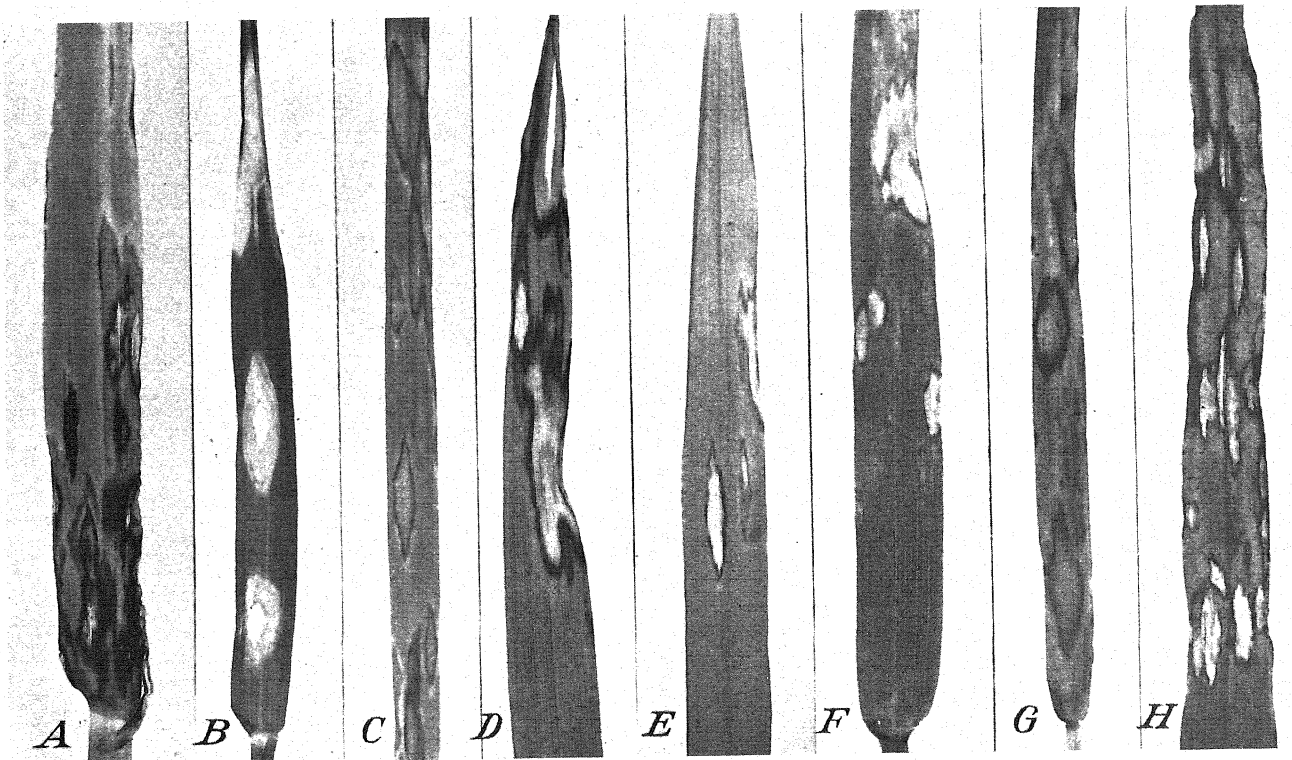
Scald as it occurs in Wisconsin, and as it has been described most frequently, is primarily a foliage disease attacking most conspicuously the blades, and to a lesser degree the leaf sheaths. The disease symptoms are strikingly similar upon all of the hosts observed by the writer (pl. 1) and may easily be recognized and distinguished from those of other leaf-spotting diseases of cereals and grasses. In the early stages of development, the lesions are of a dark bluish-gray color with a water-soaked appearance. Such areas are often 1 to 2 cm in length before evident collapse of the tissue occurs. This collapse takes place very rapidly, and in this stage, especially upon barley where large numbers of lesions are coalescing, the impression of rapid scalding is created. Lesions developing separately tend to assume a lenticular shape. The scalded area soon dries and the center assumes a light-gray color. The margin assumes a dark-brown color, which is the most distinguishing feature of the scald leaf spot. Successive enlargements of the lesions may occur and concentric brown rings result (pl. 1, *A*, *B*), giving the lesion a zonate appearance. Leaves are completely destroyed by severe infection and often almost complete defoliation of the host results.

PATHOLOGICAL HISTOLOGY

The relation of the scald fungus to the host tissue and the development of the disease have been studied microscopically upon barley leaves naturally infected in the field and artificially infected in the greenhouse. Stages in the development of the disease from penetration of the host to complete break-down of the leaf tissue and fructification of the pathogene have been followed.

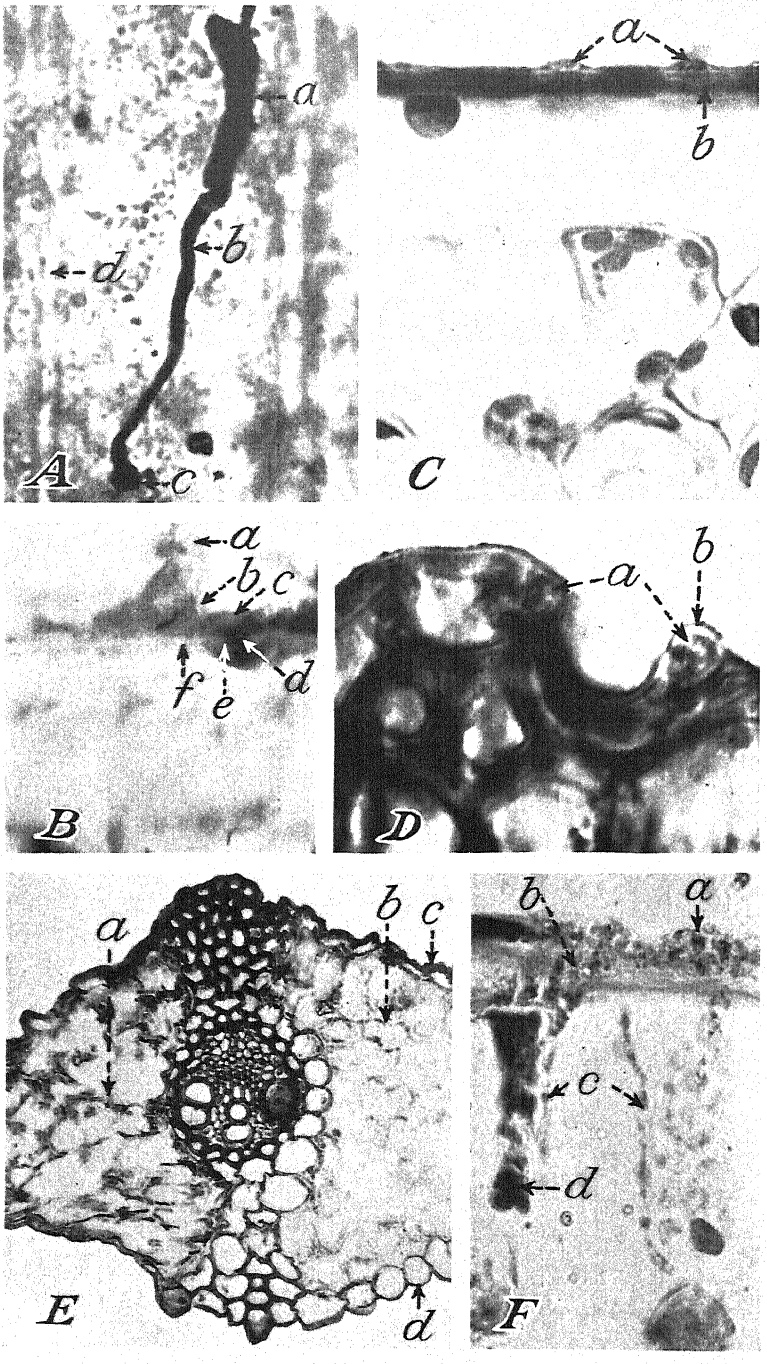
In the study use was made of both longitudinal and transverse sections of the leaf and of cleared whole leaf segments. Material for sectioning was fixed in both chrom-acetic urea and formalin-acetic-alcohol fixa-

[8] See footnote on page 177.



SYMPTOMS OF SCALD ON CEREALS AND GRASSES IN THE FIELD.

A, Barley; *B*, rye; *C*, *Bromus inermis*; *D*, *Elymus canadensis*; *E*, *Elymus virginicus*; *F*, *Agropyron repens*; *G*, *Hordeum jubatum*; *H*, *Dactylis glomerata*. *A* to *E*, natural size. *F* to *H*, × 1.5.



FOR EXPLANATORY LEGEND SEE OPPOSITE PAGE.

tives, the former being the more satisfactory. Longitudinal sections 10μ in thickness and transverse sections 20μ in thickness proved satisfactory for study. Staining in safranin followed by fast green was the

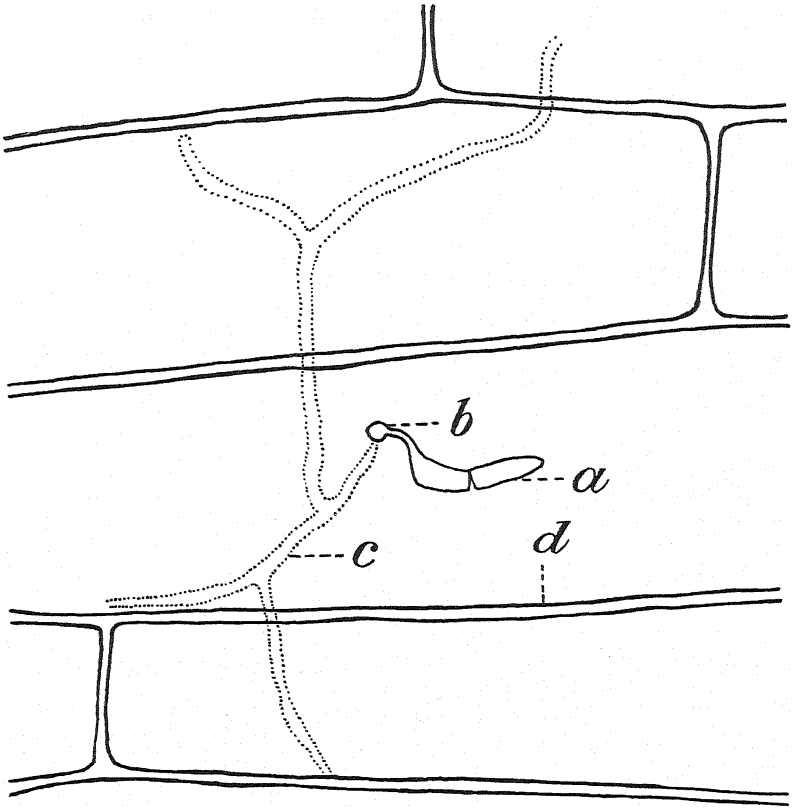


FIGURE 2.—Penetration of cuticle by *Rhynchosporium secalis* and development of subcuticular mycelium: *a*, Conidium; *b*, appressorium; *c*, subcuticular mycelium, *d*, vertical epidermal cell wall. Drawn with the aid of the camera lucida. × 700.

most useful technique used for differentiation of host and parasite tissues and pathological conditions in the host. The stages during conidium germination, appressorium formation, and after penetration

EXPLANATORY LEGEND FOR PLATE 2

A. Germinated conidium of *Rhynchosporium secalis* on a barley leaf. × 1,200. *a*, Conidium; *b*, germ tube; *c*, appressorium; *d*, vertical wall of epidermal cell.

B. Longitudinal section of seedling barley leaf showing penetration to the subcuticular position. × 1,900. *a*, Upper cell of conidium; *b*, short germ tube; *c*, appressorium; *d*, penetrating hypha; *e*, papillate growth on epidermal wall about point of fungus penetration; *f*, normal wall penciled to show clearly the normal thickness.

C. Longitudinal section of a young infection of a leaf of a nearly mature barley plant. × 1,440. *a*, Cross section of minute, slightly flattened, subcuticular hyphae; *b*, altered section of upper epidermal cell wall failing to retain safranin stain.

D. Transverse section of a collapsing lesion on a leaf of a nearly mature barley plant. × 1,800. *a*, Cross sections of subcuticular hyphae; *b*, cuticle of cell wall.

E. Transverse section of an advanced lesion on a leaf of a nearly mature barley plant. × 475. *a*, Collapsed mesophyll; *b*, normal mesophyll at margin of lesion; *c*, shows marginal advance of collapsing epidermis on dorsal surface over normal mesophyll at *b*; *d*, normal epidermis on ventral, noninfected side of leaf.

F. Longitudinal section of an advanced lesion on a leaf of a nearly mature barley plant showing penetration from the superficial stroma into the mesophyll. *a*, Superficial stroma; *b*, hyphae penetrating epidermal cell or cell wall; *c*, hyphae invading mesophyll; *d*, collapsing mesophyll cell adjacent to invading hyphae.

of the cuticle were satisfactorily studied in segments of whole leaves killed and partially cleared in a 50–50 mixture of 95-percent alcohol and glacial acetic acid, and stained in an aqueous solution of cotton blue. The conidia and germ tubes were heavily stained while the host tissue remained clear. Although the subcuticular hyphae could not be stained because of the covering of cuticle, they were clearly visible by microscopic observation, owing to light refraction.

Less complete observations of pathological histology have been made on scald of rye, *Agropyron repens*, and *Dactylis glomerata*. The macroscopic symptoms as well as internal pathology appear to be identical for scald on these hosts and on barley. Because of this similarity the scald of barley has been selected for study as a representative of the group of scald diseases, and all statements following refer specifically to scald of barley. All evidence at hand indicates that they apply also to the disease on the other hosts.

Mode of Infection

In the early stages of the development of *Rhynchosporium* in the host tissue the mycelium is wholly subcuticular. Penetration to the subcuticular position is effected within a 48-hour period. The germ tubes form very small rounded structures at the ends, apparently functioning as appressoria, from which penetration occurs (fig. 2 and pl. 2, *B*). Several appressoria may develop upon the branched germ tube system originating from a single conidium. Immediately beneath the appressoria the outer epidermal wall thickens to form a rounded papilla, several times the thickness of the wall, which projects into the lumen of the cell (pl. 2, *B*, *e*). The penetrating hypha grows into this thickening and then laterally in a subcuticular position (pl. 2, *B*, *d*, and fig. 2). Penetration may occur on either the dorsal or ventral epidermal surface.

Development of Fungus in the Host Tissue

Following penetration, the subcuticular mycelium grows rapidly and branches profusely. The first hyphae are very small in diameter and slightly flattened and oval in cross section (pl. 2, *C*, *a*). They remain very small for several days following infection. The average small diameter is about 0.6μ, while the large diameter averages about 2.2μ. After occupying a considerable area of the epidermal surface, the subcuticular hyphae enlarge (pl. 2, *D*, *a*) and impart a grayish cast to the infected area in contrast to the bright green of the surrounding tissue. The outer epidermal wall, which normally takes the safranin stain heavily, loses its affinity for this dye in the area immediately below the subcuticular hyphae (pl. 2, *C*, *b*) and becomes

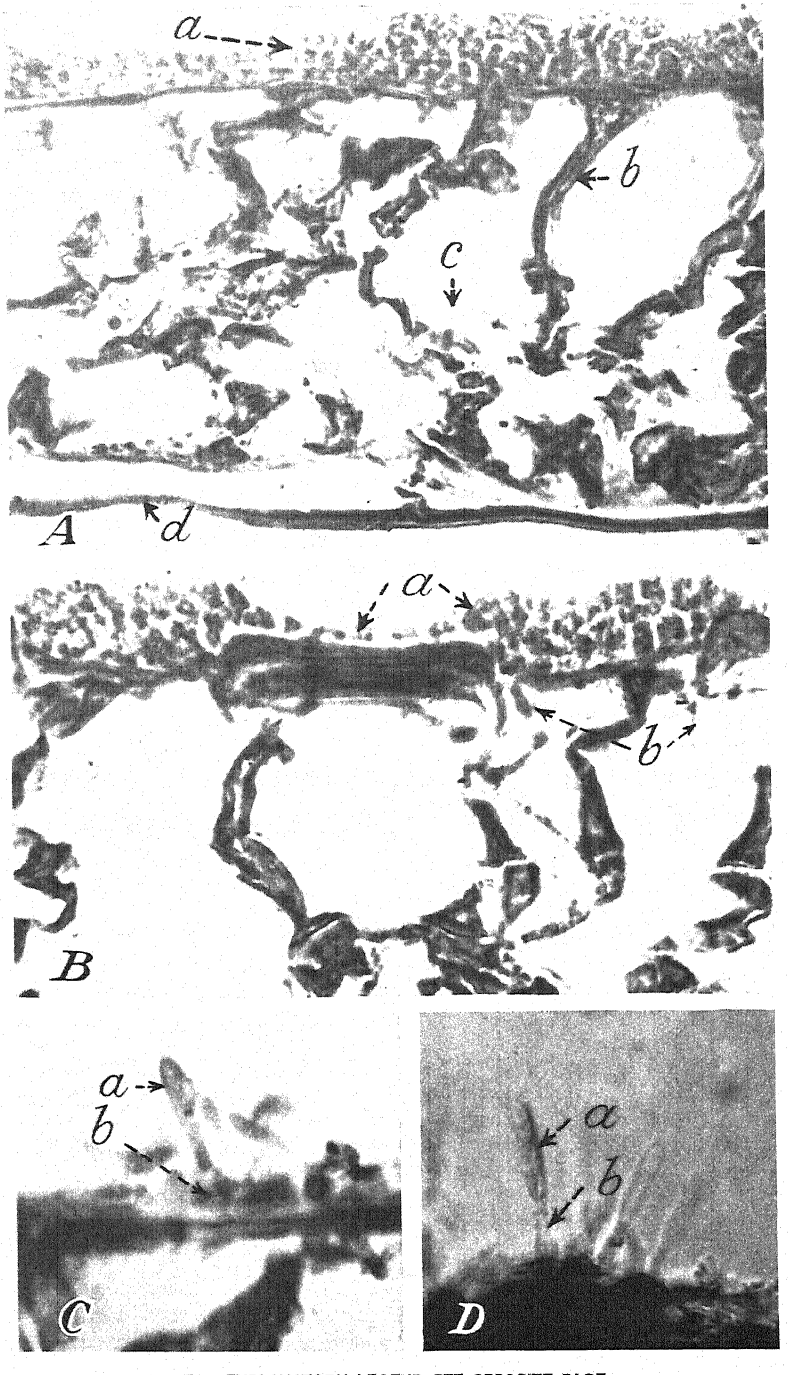
EXPLANATORY LEGEND FOR PLATE 3

A. Longitudinal section of a mature lesion on leaf of a nearly mature barley plant, showing restriction of fertile stroma to one surface of the leaf. × 700. *a*, Fertile stroma; *b*, collapsed mesophyll cell; *c*, one of the rather few hyphae occurring within the mesophyll; *d*, noncollapsed epidermal cell on surface opposite the side of infection, no stroma occurring on this surface.

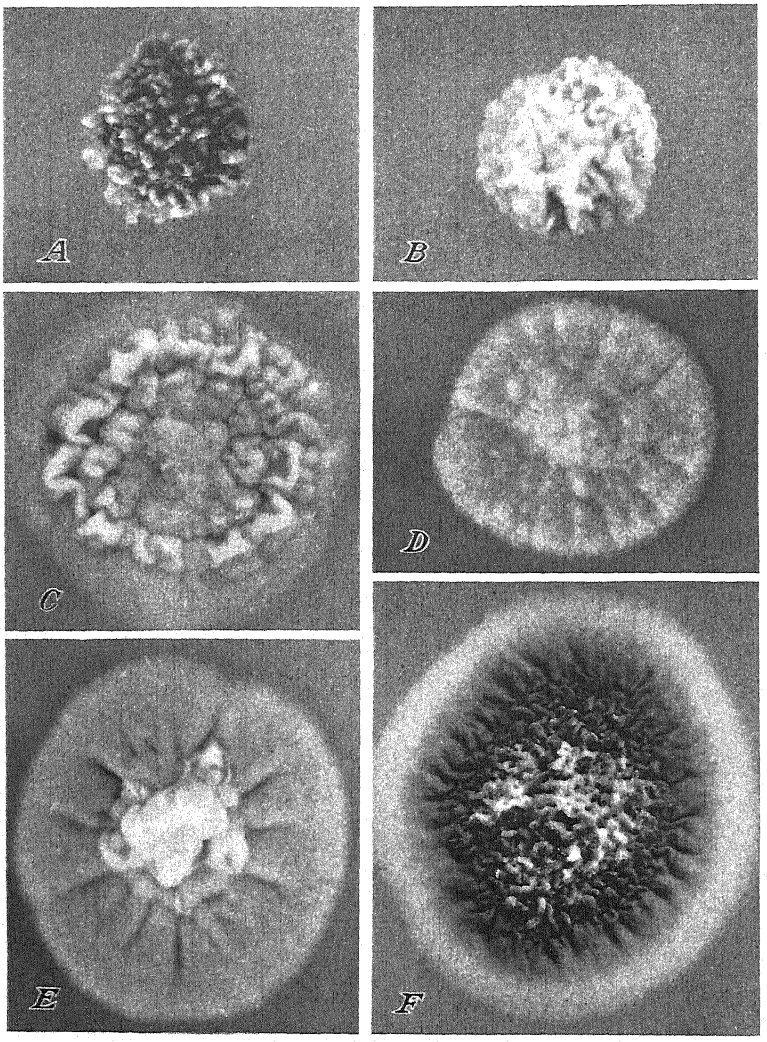
B. Longitudinal section of a mature lesion showing fertile stroma covering guard and epidermal cells. × 880. *a*, Superficial stroma; *b*, hyphae penetrating through macerated epidermal cell walls into mesophyll.

C. Longitudinal section of a mature lesion on barley, showing the distinctive manner of conidium production in *Rhynchosporium secalis*. × 1,300. *a*, Immature conidium, oriented to obscure the beaked, apical cell; *b*. cell of fertile stroma on which conidium is borne.

D. Free hand section of a lesion produced by *Rhynchosporium alismatis* (Oud.) Davis on *Alisma plantago-aquatica*, showing manner of sporulation. × 1,300. *a*, Immature nonseptate conidium (dark bands on conidium are not cross walls but stained cytoplasm, cross walls when present showing as white lines); *b*, flask-shaped conidiophore.



FOR EXPLANATORY LEGEND SEE OPPOSITE PAGE.



Photographs of 28-day-old culture of five physiologic races of *Rhynchosporium secalis* and one culture of *R. orthosporum* growing on potato 2 percent dextrose agar. × 1.75. *A*, Barley race of *R. secalis*; *B*, *Agropyron repens* race of *R. secalis*; *C*, *Elymus canadensis* race of *R. secalis*; *D*, rye race of *R. secalis*; *E*, *Bromus inermis* race of *R. secalis*; *F*, *R. orthosporum*

weakened as evidenced by frequent bending and sinking at such points. The outer epidermal wall is the first tissue to collapse, falling against the lower wall of the epidermal cell (pl. 2, *D* and *E*, *c*). Following the collapse of the epidermis, the mycelium begins to penetrate through the macerated walls of the collapsed epidermal cells into the mesophyll from the stroma which has been formed from the subcuticular hyphae (pl. 2, *F*, *b*, *c*, and pl. 3, *B*, *b*). Growth within the mesophyll is intercellular. Prior to this stage the mesophyll cells appear normal but rapidly collapse in the region of the invading mycelium (pl. 2, *F*, *d*, and pl. 3, *A*, *b*). At this stage the macroscopic symptom of scalding and water-soaking becomes evident in the leaf spots. Even after complete break-down of the mesophyll the mycelium makes only a sparse development in the interior of the leaf (pl. 3, *A*, *c*). This stage is reached about 9 days after inoculation in barley and after about 14 days in rye and the grasses under greenhouse conditions.

The infected area continues to enlarge by the radial growth of the subcuticular hyphae at its margin, the epidermis first collapsing, followed by successive penetrations of the fungus into the mesophyll (pl. 2, *E*, *c*). There is no evidence that radial growth of the intercellular mycelium within the mesophyll plays any important role as a means of enlarging the diseased area, since the subcuticular development of mycelium and subsequent epidermal collapse always considerably precede the appearance of hyphae in the underlying mesophyll at the margin of the lesion.

Fructification of the Fungus

During the invasion and break-down of the mesophyll, the subcuticular mycelium develops rapidly, covering the entire area of the leaf spot and building up a fertile stroma which may be several cells in thickness (pl. 3, *A*, *a*, and *B*, *a*). At this stage the cuticle has been pushed away from the wall and lost, leaving the stroma completely superficial. The greatest growth of the fungus is in this subcuticular and later superficial position, the mass of the fungus there far exceeding that within the mesophyll tissue. Fructification occurs after the complete break-down of the leaf tissue in the infected spot. The conidia are formed as direct outgrowths of the cells of the superficial, fertile stroma (pl. 3, *C*, *a*, *b*). Sporulation is most abundant in the central and most completely collapsed area of the leaf spot.

Owing to the restriction of the initial development of infection and subcuticular mycelium to one surface of the leaf, the fruiting stroma and sporulation are limited to that surface, although either surface may become infected (pl. 2, *E*, *c*, *d*, and pl. 3, *A*, *a*, *d*). The fungus does not grow through the leaf to fruit on the opposite surface. The fructifying surface can be identified macroscopically by the darker color of the leaf spot margin there, as contrasted with that on the opposite side. Fructification on both surfaces of a given leaf area occurs only where two separate infections take place on exactly opposite sides of the leaf.

Discussion of Pathological Histology

Penetration has been reported and figured by Bartels (*1*) as being effected primarily by the growth of conidial germ tubes through the stomata, directly into the mesophyll of the leaf. He also reported direct epidermal penetration as occurring less frequently. Mackie

(*16*) implied stomatal entry by stating that the germ tubes "form appressoria in the stomata." The observation of hundreds of germinating conidia and penetrations, in this study, has given no evidence of even the rare occurrence of stomatal entry. Germ tubes repeatedly have been observed to have grown across the surfaces of stomata to form appressoria on the upper epidermal wall elsewhere. The early stages of the pathology of the scald disease, involving abundant subcuticular development of mycelium prior to its appearance within the mesophyll tissue, precludes the possibility of stomatal entry being of significance in the initiation of infection.

Bartels (*1*) has figured the fungus as first developing within the mesophyll and later growing out to the epidermis to form the fertile mycelium within the lumen of epidermal cells of both the dorsal and ventral surfaces. Brooks (*2*), while not following penetration of the fungus, gives an account of fructification similar to that of Bartels, assuming preliminary entry of the mycelium into the mesophyll and later growth out to a subcuticular position or into the lumen of the epidermal cells where fructification occurs. Failing to understand the manner of the initiation of parasitism of *Rhynchosporium*, these workers have apparently interpreted the penetration from the subcuticular mycelium into the mesophyll as occurring in the reverse order. The writer has never observed a case where the fruiting stroma developed initially within the lumen of the epidermal cells. Occasionally, as shown in plate 2, *F*, *b*, hyphae penetrating into the mesophyll appear to have traversed the lumen of the epidermal cells. A more likely interpretation, however, is that they have grown down through the vertical wall of the epidermal cells, which are indiscernible, as is most wall tissue in the presence of masses of the fungus. It is possible that, after break-down of the epidermal cells, the fertile mycelium may develop within the macerated walls of these cells. It is certain, however, that in the writer's material, hyphae did not enter the lumen of epidermal cells preliminary to formation of the fruiting stroma.

Davis (*8*) stated that the conidia are borne on undifferentiated hyphae protruding from stomata. The papers of Frank (*11*) and Brooks (*2*) and the present work concur in showing this to be incorrect.

THE CAUSAL ORGANISM

TAXONOMY AND NOMENCLATURE

The Genus Rhynchosporium

The taxonomy and nomenclature of the genus *Rhynchosporium* have become confused in European and American literature. The first mention of the genus *Rhynchosporium* occurs in a publication by Frank (*11*) in October 1897, in which the author credits the naming of the new genus to his associate, E. Heinsen, who had not yet published his work. Heinsen (*12*) in 1901 published on this work. Both Frank and Heinsen failed to give formal descriptions of the genus or species, merely giving a general description of the fungus with illustrations. Formal descriptions were later provided by Saccardo (*18*) and Lindau (*15*), who credited authority for the genus *Rhynchosporium* and the species *R. graminicola* to Heinsen.

Davis (*8*) in 1922 emended the genus to include all Mucedinaceae, Micronemeae, Hyalodidymae. Davis conceived the conidia to be

borne in the following manner: "An undifferentiated hypha makes its way to a stoma where it bears upon its extremity a conidium." This statement has been shown to be in error by the present work and that of Brooks (*2*).

The taxonomic position of the genus *Rhynchosporium* is somewhat debatable since the genus appears to possess characters common to both the orders, Moniliales and Melanconiales. The apparent resemblance to the Melanconiales lies in the fructification from a stroma-like stratum. This stratum constitutes the main mycelial body of *Rhynchosporium*, being almost continuous over the affected portion of the leaf and more or less unlimited in its growth at its margin. The position of the stroma is essentially superficial on the leaf, although the initial growth of hyphae immediately following infection and at the margin of the stroma is in the subcuticular position. By proliferation of this mycelium the stroma is progressively built up from the center toward the margin of the leaf spot. The margin of the stroma grades into a diffuse nonstromatic mycelium, in a subcuticular position. The cuticle is soon pushed away and dislodged as the fruiting stroma is built up. Conidia are never produced within the host tissue, or even under the cuticle, later to be discharged.

In the existing keys to the genera, it is true that *Rhynchosporium* might conveniently be placed in the order Melanconiales or even considered synonymous with *Marssonina*. However, this study is interpreted as giving little evidence that *Rhynchosporium* is closely related to genera of this order. If such an interpretation were made, the whole more or less diffuse and superficial fungus body, which is often over 1 inch long and one-half inch wide, must be considered a single acervulus. Therefore, as the preferable alternative, the fruiting structure is here interpreted as a more or less compact, yet little organized mycelium, from which conidia are directly abstricted as in many other genera of the Moniliales. On the basis of these considerations *Rhynchosporium* is here recognized as a valid genus in the family Moniliaceae.

Frank's illustrations indicate that he correctly understood the morphology of the fruiting structures of the genus. However, the descriptions by Saccardo and Lindau are based on Heinsen's studies of the morphology of the fungus in culture and are misleading as to the morphology of the fructifying structure on the host, which, in the writer's opinion, is the important distinguishing feature of the genus. It seems necessary, therefore, to present here an emended description of the genus, including the characteristic features of fructification.

Rhynchosporium Heinsen.

Parasitic, producing spots on leaves; sterile mycelium sparse in mesophyll of host; mycelium subcuticular at first, later developing into a superficial fertile stroma more or less covering the leaf spot; conidiophores absent; conidia one-septate, hyaline, sessile on cells of fertile stroma.

The emended genus, *Rhynchosporium*, would fall in the classification, Moniliaceae, Hyalodidymae, Micronemeae.

Nomenclature of Rhynchosporium secalis (Oud.) Davis

The first published record of the scald fungus was made by Oudemans (*17*) in June 1897, under the binomial *Marsonia secalis*, 4 months previous to Frank's publication (*11*) on the same organism under the name *Rhynchosporium graminicola* Heinsen. Davis (*7*), in 1919, believing that Oudemans had incorrectly classified the fungus in the

Melanconiales, recognized Heinsen's genus *Rhynchosporium* in making the new combination *Rhynchosporium secalis* (Oud.) Davis.

Bartels (*1*), in 1928, made the combination *Marssonina graminicola* (Ell. and Ev.) Sacc., placing *Gloeosporium graminicolum* Ell. and Ev., *M. secalis* Oud., and *Rhynchosporium graminicola* Frank in synonymy. He recognized as having priority a description by Ellis and Everhart (*10, p. 154*) of *G.* (*Marsonia*) *graminicolum*, collected upon an unidentified grass by John Dearness at London, Canada. The description given of *G. graminicolum* has nothing in common with the characters of the scald fungus. Through the efforts of J. J. Davis, cotype material of *G. graminicolum* Ell. and Ev. has been secured from Dearness for study by the writer. This specimen is not the scald fungus and shows no similarity to it either in disease symptoms or fungus structure, and therefore can have no priority in the nomenclature. Ellis and Everhart's report (*10*) is accurately descriptive of the specimen. The internal morphology of the host strongly suggests it to be one of the Cyperaceae.

In view of the priority of Oudemans' description, the valid name of this species, then, is *Rhynchosporium secalis* (Oud.) Davis.

New Species

A new species of the genus *Rhynchosporium* has been collected in Wisconsin by the writer (*4*) and by Sprague [10] in Oregon, attacking *Dactylis glomerata*. This fungus produces symptoms identical with those of *R. secalis*. The fruiting structure is likewise similar. The new species, here designated as *R. orthosporum*, has uniformly cylindrical conidia which distinguish it from *R. secalis* (fig. 1). It is described below.

Rhynchosporium orthosporum sp. nov.

In foliis; maculis amphigenis, 0.5–3.0 cm longis, lenticularibus et saepe confluentibus, initio humido-fusco-olivaceis, margine concolori, dein centro canis, brunneo-marginatis; conidiis 14.4–19.4 × 2.3–4.7 μ, erectis, clyindraceis, medio 1-septatis, in stromatibus superficialibus sine basidiis et plus minusve maculas omnino occupantibus.

Hab. in foliis *Dactylidis glomeratae* in Wisconsin, 1929.

Rhynchosporium orthosporum sp. nov.

On leaves; spots, 0.5–3.0 cm long, lenticular in shape, coalescing to form irregular lesions, at first water-soaked, dark olivaceous, later becoming gray surrounded by a brown margin; conidia 14.4–19.4 × 2.3–4.7 μ, erect, cylindrical, medianly septate, formed directly on cells of superficial stroma, more or less covering leaf spot.

On *Dactylis glomerata* L., Whitewater, Wis., May 11, 1929, Caldwell (type); also from other locations in Wisconsin and from Benton County, Oreg.

Cotype specimens have been deposited in the herbarium of the University of Wisconsin and in the mycological collections of the Bureau of Plant Industry, United States Department of Agriculture.

Excluded Species

Davis (*8*) has made the combination *Rhynchosporium alismatis* (Oud.) Davis, placing *Septoria alismatis* Oud., *Aschochyta alismatis* Ell. and Ev., *Ramularia alismatis* Fautr., and *Didymaria aquatica* Starb., occurring on *Alisma* and *Sagittaria*, in synonymy with it.

[10] Sprague, R. See footnote 8.

Through the kindness of Dr. Davis, a fresh specimen of *Rhynchosporium alismatis* (Oud.) Davis on *Alisma plantago-aquatica*, collected by him at Klevensville, Wis., on July 12, 1929, was made available for study. Freehand and paraffin sections of this specimen show this fungus to lack the superficial fertile stroma and to bear the conidia on short flask-shaped conidiophores on the leaf surface (pl. 3, *D*). Thus, this species does not fall in the division Micronemeae as stated by Davis (*8*). The presence of conidiophores together with the lack of the superficial stroma excludes this species from the genus *Rhynchosporium*.

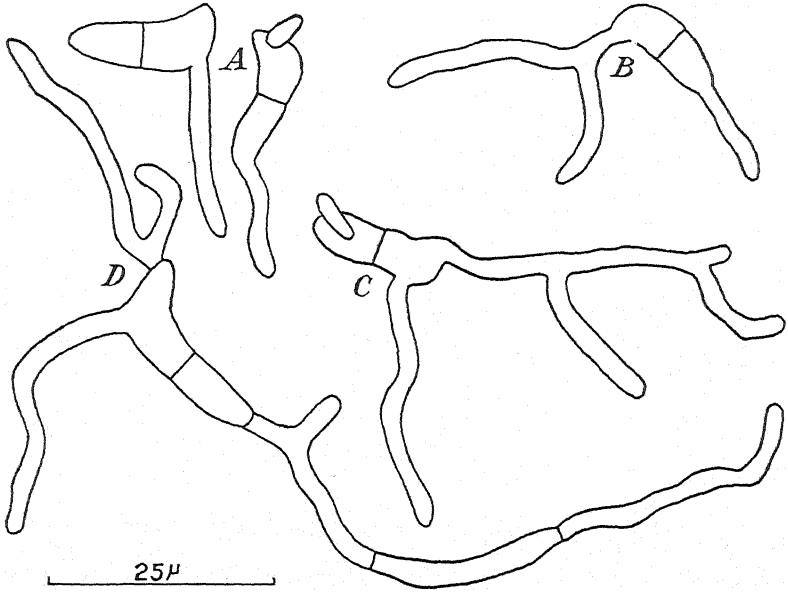


FIGURE 3.—Germination in water of culture-grown conidia of barley race of *Rhynchosporium secalis*: *A* to *D* represent growth stages after 8, 12, 24, and 36 hours, respectively.

Thus the genus as here defined includes only the species *Rhynchosporium secalis* (Oud.) Davis and *R. orthosporum* sp. nov.

ISOLATION AND CULTURE

Cultures of the scald organism from the several hosts may usually be obtained by the planting of small pieces of diseased leaf tissue on potato-dextrose agar after surface sterilization. Surface sterilization was accomplished by dipping the leaf pieces into 70-percent alcohol for 20 seconds, removing them to 1 to 1,000 mercuric bichloride solution for 1 to 2 minutes, and transferring them to agar plates after washing in sterile water. The fungus grows very slowly and requires 4 or 5 days to become macroscopically evident. Isolation during the warm summer months at Madison, Wis., is often difficult since the lesions are frequently overrun by various other fungi, predominantly *Alternaria* spp., which completely suppress the development of *Rhynchosporium*. When conidia from the host are available, cultures may be secured by pouring suspensions of conidia in

sterile water over agar plates and draining off the excess moisture. Isolated germinating spores usually must be located under the microscope and removed to agar slants soon after germination to avoid contamination.

The conidia of *Rhynchosporium secalis* and of *R. orthosporum* germinate upon agar within a 24-hour period. The details of the process, in the case of *R. secalis*, have been well described by Heinsen (*12*). The upper cell usually germinates first, with the germ tube swelling from the side of the cell or tip of the beak. The lower cell may then send out a tube. Either or both cells commonly produce a second tube after elongation of the first. Conidia germinating in distilled water are shown in figure 3. In water at 20° C., germination begins within 4 hours. The contents of the conidia, at first quite homogeneous, become vacuolate preceding germination. On agar, germination is initiated more tardily and germ tubes elongate more slowly than in distilled water.

Growth Response upon Different Media

All the races of *Rhynchosporium secalis* as well as the species *R. orthosporum* grow slowly in culture. The most rapid growth, on the media used, occurred upon potato-dextrose agar. The rye and barley races of the fungus have been cultured upon a variety of media including barley and rye-leaf-decoction agars, corn meal, and corn meal 1-percent dextrose agars, lima bean agar, oatmeal agar, potato, potato 1-percent dextrose, and potato 2-percent dextrose agars, 1- and 2-percent malt extract agars, and upon sterilized barley stems. The volume of mycelium and spores produced, within this range of media, apparently varied directly with the concentration of dextrose or soluble carbohydrate present. Judging from the conformation of the conidia, the most nearly normal development occurred on those media with low sugar content, i. e., corn meal, oatmeal, and lima bean agars, where conidia not unlike those from the host were produced (fig. 1). Conidia from potato dextrose and malt agars were of variable shape and appeared to be little more than undifferentiated terminal or branch cells of the fertile hyphae.

Temperature and Humidity Relations

Conidial Germination and Germ-Tube Growth

To improve the inoculation technique, an experiment was conducted to determine the relation of temperature to spore germination and germ-tube elongation. Conidia of a culture isolated from barley were washed into sterile distilled water from 7-day-old corn meal-agar slant cultures. Drops of the spore suspension were transferred by a platinum loop to carefully cleaned cover glasses and were inverted as hanging drops over unsealed glass rings in Petri dishes. The bottoms of the Petri dishes were moistened with water. A selected standard-size loop was used to transfer all drops, giving them a uniform size. The hanging-drop, conidial suspensions were then incubated at temperatures ranging from 2° to 35° C. At certain intervals of time 25 spores were selected at random near the periphery of the drop and the longest germ tube of each spore was measured. Observations were continued for 48 hours. However, after 36 hours

branching of the germ tubes had become so profuse as to make measurements of germ-tube length unreliable as an index of growth. Only the germ tubes of conidia near the periphery of the drops were observed, since germination was more vigorous there. The data from this experiment are presented in figure 4. These curves show the minimum temperature for germ-tube growth in distilled water to lie between 2° and 4°, the optimum between 18° and 21°, with the maximum between 28° and 30°. The sudden reduction in rate of growth between 21° and 24° is striking. Similar reactions were evident in a number of germination trials where no measurements were made. The same temperature relation was observed in the case of the conidia from a culture isolated from rye but a more tardy germination occurred at all temperatures.

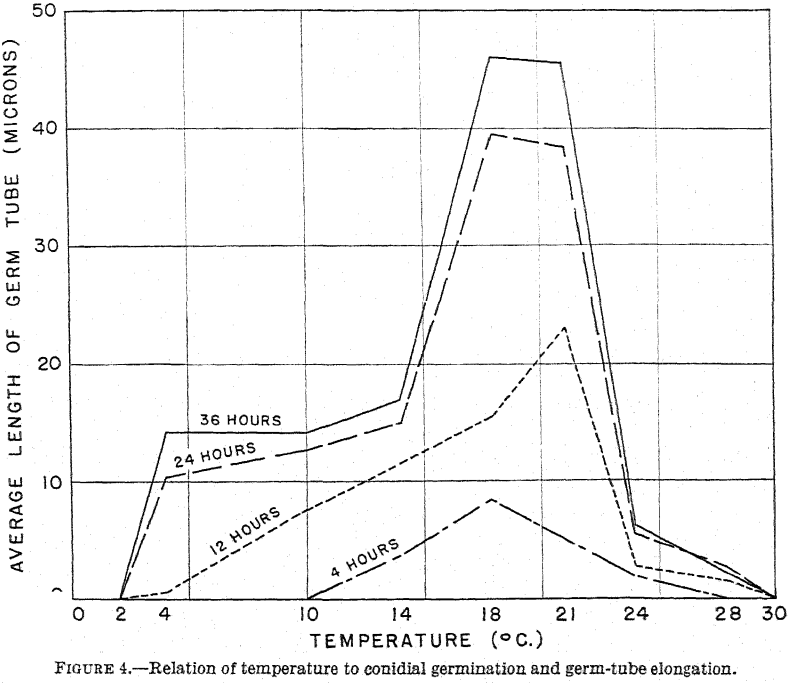


FIGURE 4.—Relation of temperature to conidial germination and germ-tube elongation.

When conidia in water are subjected to temperatures of 30° C. and above, the contents usually become vacuolate and one or both cells rupture, extruding their protoplasm into the surrounding liquid. Some of the spores remain intact at 30° but at higher temperatures (32° and 35°) practically all of them rupture. As stated above, conidia in hanging drops were held at the several temperatures for 48 hours. The conidia failing to germinate at 2°, 30°, 32°, and 35° were then placed in a 20° chamber. The spores formerly held at 2° now promptly germinated. A few of the conidia that had not ruptured at 30° also germinated. Those held at 32° and 35° were all dead. Those spores which germinated slowly at 4° were held at that temperature over a period of several days and were observed to continue to develop to a degree which would seem sufficient to

produce infection. This is of interest in view of the fact that the disease makes very rapid development on barley in the fall when temperatures are near and below freezing for periods of considerable duration.

Sporulation on the Host

The development of scald following artificial inoculation proceeds normally in the greenhouse during the winter under controlled temperatures ranging from 12° to 24° C. Leaf lesions typical of the disease are produced, but the fungus failed to produce conidia in appreciable numbers on all such lesions examined. It was found that the limiting factor in conidial production under greenhouse conditions was apparently the low humidity, for when plants bearing sterile lesions were placed in a glass-moist chamber of 95-percent humidity and controlled temperature the lesions became abundantly covered with conidia, while controls in the open house at the same temperature failed to fruit.

The production of conidia upon the host also seems to be affected by temperature. This relation was tested by placing diseased barley plants, found to be free of conidia, in a series of moist chambers (approximately 95-percent humidity) held at temperatures of 5°, 10°, 15°, 20°, and 30° C. After a period of 48 hours' exposure to these temperatures the lesions were examined for conidia. The results, although not quantitative, were striking. At 5° and 10° numerous conidia were produced, yet they were relatively few in contrast to the myriads to be obtained from plants held at 15° and 20°. At 30° no conidia could be found; however, at this temperature and high humidity the lesions were overrun by various saprophytes which may have influenced the scald organism. These temperature relations agree quite closely with those for germ-tube elongation, where the fungus is wholly inactive at 30° and, although somewhat retarded, effectively active at 5°.

Physiologic Specialization

Material and Methods

The inoculum (conidia and mycelial fragments) for most of the experiments reported here was secured from cultures of the organism on potato-dextrose agar slants. Except for one culture mentioned later, only monosporous cultures were tested. The conidia, which are formed in masses intermixed with mycelium, cannot readily be washed from the tubes. Therefore, the fungus mass was removed from the tubes, crushed to fine fragments with a spatula, then taken up with water and strained through cheesecloth to remove the larger fragments of mycelium. The inoculum was sprayed over the plants with an atomizer and allowed to dry. After drying of the inoculum, the plants tested in the greenhouse were reatomized with tap water and placed in a moist chamber held at 100-percent humidity and a temperature of about 20° C. The foliage was kept continuously wet for 48 hours, after which the plants were removed to the greenhouse bench. In some tests conidial inoculum was washed from diseased host plants. The sources of cultures used in these studies are given in table 1.

TABLE 1.—*Cultures of Rhynchosporium secalis and R. orthosporum tested in cross-inoculation studies, with host source and place of collection*

Species and culture no.	Host source	Place of collection	Date of collection	Collector
R. secalis:				
B11	Barley	Madison, Wis.	October 1926	Writer.
B21	do	do	October 1927	Do.
B31	do	do	do	Do.
B42	do	Corvallis, Oreg.	December 1928	H. P. Barss.
B51	do	Madison, Wis.	June 1929	Writer.
B62	do	do	November 1929	Do.
B71	do	Corvallis, Oreg.	March 1931	R. Sprague.
B82	do	Madison, Wis.	July 1932	Writer.
B10	do	Germany		H. Klebahn.
Hm11	*Hordeum murinum*	Corvallis, Oreg.	February 1934	R. Sprague.
Hj11	*H. jubatum*	Madison, Wis.	July 1935	Writer.
A13	*Agropyron repens*	do	October 1928	Do.
A2	do	do	June 1929	Do.
A41	do	Middleton, Wis.	July 1932	Do.
Br. 14	*Bromus inermis*	Madison, Wis.	October 1928	Do.
Br. 21	do	do	May 1929	Do.
Br. 51	do	do	June 1929	Do.
E. c. 13	*Elymus canadensis*	do	October 1928	Do.
E. c. 21	do	Fort Atkinson, Wis.	May 1929	Do.
E. c. 31	do	Madison, Wis.	June 1930	Do.
R. 11	Rye	do	October 1926	Do.
R. 21	do	do	October 1927	Do.
R. 31	do	do	May 1929	Do.
R. 41	do	do	May 1930	Do.
R. orthosporum:				
D11	*Dactylis glomerata*	do	May 1927	Do.
D21	do	Whitewater, Wis.	May 1929	Do.

The cross inoculations in the field were conducted similarly. After spraying the plants with inoculum from cultures they were covered for a 48-hour period with muslin tents, which were kept moist with a continuous fine spray of water, after which the tents were removed. Only seedling plants were tested in the field.

The host plants were inoculated in the greenhouse in various stages of maturity, ranging from seedling to flowering plants. The degree of maturity of the plants from the seedling to the flowering stage apparently has no effect on susceptibility of greenhouse or field-grown plants. No attempt was made to maintain a definite number of plants of a host species in the different trials. Generally from three to several plants, growing in one or more 4-inch pots, represented a host species in each inoculation series.

Symptoms on barley appear earlier than on the other hosts and are at maximum development 2 weeks after inoculation. On rye and on species of *Agropyron*, *Bromus*, *Elymus*, *Dactylis*, and *Hordeum* 3 weeks or longer may be required for full expression of the disease. Observations for infection were made at approximately weekly intervals, beginning 14 days after inoculation and continuing for 4 weeks.

The cross-inoculation studies have involved each of the host species from which cultures of the scald organism had been isolated by the writer as well as other species of the same genera and a number of species of other genera from which no cultures were available. The host species from which the cultures used in these studies were isolated serve to distinguish the physiologic races of the scald organism. Seed of the host species dealt with in table 2 were collected in the vicinity of Madison, Wis., or La Fayette, Ind.

TABLE 2.—*Greenhouse cross-inoculation studies with Rhynchosporium secalis and R. orthosporum* [1]

RHYNCHOSPORIUM SECALIS

Host[2] source of culture	Culture[3] designation	Date of inoculation	Results on differential hosts: Barley [4]	Rye [4]	*Agropyron repens*	*Bromus inermis*	*Dactylis glomerata*	*Elymus canadensis*	*Hordeum jubatum*	*Hordeum murinum*
Barley	B11	Jan. 15, 1927	+	−						
	B31	Feb. 23, 1929	+	−	−	−	−	−	−	
	B31	Apr. 6, 1934	+	−	−	−	−	−	−	−
	B31 [5]	May 14, 1934	+	−	−	−	−	−	−	−
	B42	Feb. 14, 1929	+	−		−	−			
	B62	Mar. 15, 1930	+	−		−	−	−	−	
	B71	Apr. 1, 1931	+	−	−	−	−	−	−	
	B71	Apr. 10, 1934	+	−	−	−	−	−	−	−
	B82	Mar. 11, 1933	+	−	−	−	−	−	−	−
	B10	Apr. 11, 1934	+	−	−	−	−	−	−	−
	B10	May 18, 1934	+	−	−	−	−	−	−	−
Hordeum murinum	H.m. 11	Apr. 9, 1934	+	−	−	−	−	−	−	−
	H. m. 11	Apr. 29, 1934	+	−	−	−	−	−	−	+
	H. m. 11	Apr. 17, 1935	+	−	−	−	−	−	−	+
	H. m. 11	Feb. 15, 1936	+	−	−	−	−	−	−	−
Hordeum jubatum	H. j. 11	May 6, 1931	−	−	−	−	−	−	+	
	H. j. 11	Feb. 27, 1933	−	−	−	−	−	−	+	−
	H. j. 11	May 10, 1934	−	−	−	−	−		+	−
	H. j. 11 [6]	May 12, 1934	−	−	−	−	−	−	+	−
Agropyron repens	A 13	Feb. 25, 1929	−	−	+	−	−	−	−	
	A 13	June 26, 1934	−	−	+	−	−	−	−	−
	A 2	Mar. 12, 1930	−	−	+	−	−	−	−	
	A 2	May 2, 1931	−	−	+	−	−	−	−	
	A 41	Jan. 12, 1933	−	−	+	−	−		−	−
	A 41	Apr. 21, 1934	−	−	+	−	−	−	−	−
	A 41	May 19, 1934	−	−	+	−	−	−	−	−
Bromus inermis	Br. 14	Mar. 7, 1929	−	−	−	+	−	−	−	
	Br. 21	Mar. 10, 1930	−	−	−	+	−	−	−	
	Br. 51	Feb. 18, 1932	−	−	−	+	−	−	−	−
Elymus canadensis	E. c. 13	Mar. 19, 1929	−	−	−	−	−	+	−	
	E. c. 21	Feb. 14, 1930	−	−	−	−	−	+	−	−
	E. c. 31	Feb. 24, 1932	−	−	−	−	−	+	−	−
	E. c. 31	Feb. 21, 1933	−	−	−	−	−	+	−	−
Rye	R 11	1929	−	+	−	−	−	−	−	
	R 21	Mar. 7, 1920	−	+	−	−	−	−	−	
	R 41	Feb. 28, 1931	−	+	−	−	−	−	−	

RHYNCHOSPORIUM ORTHOSPORUM

Dactylis glomerata	D 11	Mar. 17, 1929	−	−	−	−	+	−	−	
	D 11	Apr. 16, 1934	−	−	−	−	+	−	−	−
	D 21	Mar. 17, 1930	−	−	−	−	+	−	−	
	D 21	Apr. 4, 1934	−	−	−	−	+	−	−	−

[1] Infection is indicated by a plus (+) sign; failure to infect by a minus (−) sign; no test made is indicated by leaders.
[2] All inoculum was produced in potato-dextrose agar culture except as stated in footnotes.
[3] All cultures except A 2 were derived from isolated single conidia.
[4] Oderbrucker barley (Wisconsin Pedigree 6) and Schlanstedt rye were used in tests made during the period 1927–30 and Oderbrucker (C. I. 182) and Rosen rye during the period 1931–35.
[5] Inoculum washed from leaves of infected barley.
[6] Inoculum washed from leaves of infected *Hordeum jubatum*.

RESULTS OF CROSS INOCULATION TRIALS

From tables 2 and 3 it is apparent that the cultures of *Rhynchosporium secalis* tested in this study represent six highly specialized physiologic races which can be distinguished by their ability to attack the six hosts rye, barley, *Agropyron repens*, *Bromus inermis*, *Elymus canadensis*, and *Hordeum jubatum*.

The race of *Rhynchosporium secalis* attacking cultivated barley (*Hordeum vulgare*) is represented here by a total of eight cultures from the States of Wisconsin and Oregon and one from Germany. In

these tests this race has been unable to attack any of the species inoculated except barley. No variation in host specialization occurred among the several cultures of this race. Two of them, however, cultures B71 from Wisconsin and B10 from Germany, produced symptoms more slowly than the others and fruited sparingly, while the others fruited abundantly when exposed to high humidities for 24 hours. The culture from *H. murinum* is apparently the same or a closely related form, appearing identical in its effect on barley and in addition attacking *H. murinum* in two of the four tests conducted. It is likewise similar to the barley race in culture. Neither the cultures from barley nor those from *H. murinum* were able to attack *H. nodosum* or *H. pusillum*. The field inoculation tests with the barley race are in agreement with the greenhouse tests. The barley varieties Oderbrucker and Wisconsin Pedigree 39 were susceptible to two cultures of the barley race, while two varieties each of rye, oats, and wheat were immune.

TABLE 3.—*Field cross-inoculation studies with Rhynchosporium secalis*

Grain and variety inoculated	Results with cultures from—							
	Barley				Rye			
	B21		B51		R21		R31	
	Leaves inoculated	Leaves infected	Leaves inoculated	Leaves infected	Leaves inoculated	Leaves infected	Leaves inoculated	Leaves infected
	Number	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*
Barley:								
Oderbrucker	168	63	315	173	159	0	279	0
Wisconsin Pedigree 39	183	31	198	110	159	0	219	0
Rye:								
Schlanstedt	297	0	396	0	210	25	418	42
Rosen	288	0	330	0	189	38	366	39
Oats:								
Kherson	198	0	258	0	192	0	197	0
Swedish Select	183	0	174	0	171	0	240	0
Wheat:								
Marquis	192	0	278	0	231	0	276	0
Turkey	237	0	357	0	135	0	246	0

An experiment on overwintering of the scald organism reported below (table 4) provided another test of specialization of the barley race. In April and May 1927, barley and rye were exposed in a moist chamber to dead scald-infected leaves of barley that had lain in the open during the winter. This exposure resulted in a heavy infection of the barley and no infection of the rye.

A specific race of *Rhynchosporium secalis*, as represented by one culture from Madison, Wis., occurs on *Hordeum jubatum*. This race is distinct from that on cultivated barley and *H. murinum*. It attacked neither of these hosts nor any of the other hosts, here considered as differential species. It is, however, very aggressive in its attack of *H. jubatum*. It also attacks *H. nodosum* and *H. pusillum*.

A distinct race of *Rhynchosporium secalis* attacking rye is represented in these studies by three cultures, all collected near Madison. This race is able to attack only rye among the differential hosts. No other host species has been found to be susceptible. The field inoculations

with two cultures of this race showed Schlanstedt and Rosen rye to be susceptible, while two varieties each of barley, oats, and wheat were immune.

Agropyron repens is likewise attacked by a distinct specialized race of the scald fungus. Three cultures, two of which came from the vicinity of Madison and one from Middletown, Wis., represent the race. This race also attacks *A. tenerum* but none of the other differential species.

Three cultures from *Elymus canadensis* represent a distinct race of *Rhynchosporium secalis* attacking *E. canadensis* but none of the other differential species. *E. virginicus*, *E. striatus*, and *Agropyron tenerum* were also infected. This race is the only one studied which was found to be capable of attacking a host species of a genus other than that from which it was isolated.

TABLE 4.—*Scald infection resulting from contact of seedling barley and rye with dead overwintered, scald-infected barley plants*

Host plants exposed	Leaves exposed	Leaves infected	
	Number	*Number*	*Percent*
Oderbrucker barley	221	165	74.7
Schlanstedt rye	177	0	0.0

A separate race of *Rhynchosporium secalis* is specialized on *Bromus inermis* and other species of *Bromus*. Three cultures from the vicinity of Madison represent this race. While failing to attack any of the differential species other than *B. inermis*, this race has produced infection on *B. arenarius*, *B. lanuginosus*, *B. madritensis*, and *B. villosus*.

Two cultures of *Rhynchosporium orthosporum*, isolated from *Dactylis glomerata* collected at Madison and Whitewater, Wis., have been tested for pathogenicity on the differential hosts of the six races of *R. secalis* (table 2). Like the races of *R. secalis*, it shows a high degree of host specificity, being unable to attack any variety inoculated other than *D. glomerata*.

A number of additional species reported to be susceptible in Germany by Bartels (*1*), but from which no cultures were available, have been included in these inoculation studies with negative results. This group includes the species *Cynosurus cristatus*, *Holcus lanatus*, *Lolium italicum*, *L. perenne*, *Phleum pratense*, and *Poa pratensis*. These species proved to be immune to one or more cultures of each of the races of *Rhynchosporium secalis* included in this study with the exception of *C. cristatus*, which was not tested to the *Bromus* race. Bartels (*1*) also found *Agrostis stolonifera* to be susceptible, while the closely related or synonymous species, *A. alba*, was immune from all of the races tested in the present work.

CULTURAL CHARACTERS OF PHYSIOLOGIC RACES

One representative culture of each of five physiologic races of *Rhynchosporium secalis* and one of *R. orthosporum* have been compared in Petri dish cultures. Cultures of the race on *Hordeum jubatmu*

were not available when this comparison was made. The media used were potato-dextrose and malt agar containing 1.5 percent of Bacto agar and 2 percent of dextrose and malt extract, respectively. Each plate contained 40 cc of the medium. The plates were inoculated in the centers with approximately equal masses of mycelium from monosporous cultures of the several races growing on malt agar.

The type of growth upon the two media was similar for any one race, although all races grew more rapidly on the potato-dextrose agar. Two general types of growth were to be distinguished among the six cultures (pl. 4). *Rhynchosporium orthosporum* and races of *R. secalis* from rye, *Bromus inermis*, and *Elymus canadensis* grew out from the inoculum center in a uniform radial manner, while races from barley and *Agropyron repens* heaped up to form irregular masses of mycelium and conidia. Differences in pigmentation were marked between the several races. The colonies of the barley race soon darkened and within 2 weeks were almost black. The race from *A. repens* was quite similar to that from barley but retained a light-pink cast. Colonies of the races from *E. canadensis* and rye remained light in color, while the colony from *B. inermis* became brown and produced a brown pigment which stained the medium about the colony. *R. orthosporum* soon developed a very dark pigment. These characters are relatively constant for each race as described and appeared in all isolations obtained.

Differences in abundance of fructification are marked and constant between certain races, but this character may vary in different cultures of other races. In the variable races a culture may change from fruitfulness to near sterility or the reverse. The barley race is uniformly highly conidial and has never deviated from this character. Cultures of the *Agropyron* race were similar in this respect although somewhat less fruitful. The race from rye is distinguished by the production of very few conidia in all cultures obtained. The races from *Elymus canadensis* and *Bromus inermis* are notable for their variability, some cultures producing abundant conidia and others none. Furthermore, a fruitful culture of these races may change to almost complete sterility. Attempts to influence fruitfulness in cultures by variations in nutrients and pH of media, temperature, and light have been unsuccessful.

Morphology of Conidia of Physiologic Races

A comparative study has been made of the shape and size of the conidia of six physiologic races of *Rhynchosporium secalis* and of *R. orthosporum* as produced upon the host in nature and in culture. Fifty conidia of each race from the host, and the same number from 7- to 10-day-old, simultaneously grown, corn meal agar cultures, were measured and morphologically compared. Only mature conidia were considered, clearly evident septation being the criterion of maturity.

The conidia of the several races from the hosts proved to have no distinguishing features either in shape or size (table 5 and fig. 1). Thus *Rhynchosporium secalis* is a relatively homogeneous species as far as conidia from the hosts are concerned.

TABLE 5.—*Measurements of conidia from host plants and cultures of races of Rhynchosporium secalis and of R. orthosporum*"[1]

Species of fungus and hosts from which conidia and cultures were obtained	Length						Diameter					
	Maximum		Minimum		Mean±S.E.		Maximum		Minimum		Mean±S.E.	
	Host	Culture	Host	Culture	Host	Culture	Host	Culture	Host	Culture	Host	Culture
R. secalis:	μ	μ	μ	μ	μ	μ	μ	μ	μ	μ	μ	μ
Agropyron repens	18.9	16.2	11.7	9.7	15.0±0.28	13.2±0.28	3.6	4.7	2.2	2.5	2.7±0.07	3.8±0.10
Bromus inermis	18.4	21.6	13.0	16.7	16.9± .24	18.3± .17	4.7	4.9	2.9	2.7	4.0± .07	3.5± .06
Elymus canadensis	18.0	18.0	12.1	12.6	15.1± .32	14.2± .24	5.4	3.8	2.7	1.4	3.9± .02	3.1± .15
Hordeum jubatum	19.8	----	12.6	----	16.3± .04	----------	3.6	----	2.7	----	2.9± .08	----------
Barley	21.6	21.6	12.6	13.5	15.8± .21	17.1± .28	3.8	4.9	2.7	2.5	3.3± .05	3.2± .08
Rye	19.8	17.3	10.8	9.0	16.4± .27	12.7± .05	3.6	5.4	2.3	3.1	2.8± .11	4.1± .15
R. orthosporum:												
Dactylis glomerata	19.4	19.8	14.4	11.7	16.9± .18	16.0— .28	4.7	4.3	2.3	2.5	3.0± .03	3.1± .16

[1] Measurements were of 50 spore samples of conidia from both host and cultures. Conidia were produced in corn meal-agar cultures 6 to 10 days old.

This was not true, however, of conidia from culture among which marked and consistent differences between some races were evident. Thus the mean lengths of conidia of the barley and *Bromus inermis* races were 17.1μ and 18.3μ, respectively, while those of the *Agropyron repens* and rye races were 13.2μ and 12.7μ, respectively. The situation is reversed relative to diameter, those of the rye and *A. repens* races exceeding those of the barley and *B. inermis* races.

DISCUSSION OF PHYSIOLOGIC SPECIALIZATION

Two workers in Germany have published the results of cross-inoculation studies with *Rhynchosporium secalis*, indicating that collections of this organism from several host species in Germany display no differences in host specialization. Heinsen (*12*) sprayed barley, rye, wheat, and oats with a conidial suspension of *R. secalis* both in the field and greenhouse and obtained heavy infection of barley and rye, light infection of wheat, and no infection of oats. The source of his conidia was not given. Therefore one cannot be certain that he was not dealing with a mixture of races. He reports, however, one apparent case of spread of scald from rye to barley in field plots. Bartels (*1*) conducted cross inoculations involving conidial inoculum taken directly from barley, rye, *Hordeum murinum*, and *Lolium perenne*, with which he inoculated the cereal hosts barley, rye, wheat, and oats, and grass hosts of the genera *Agropyron*, *Agrostis*, *Bromus*, *Cynosurus*, *Hordeum*, *Lolium*, *Holcus*, and *Phleum*. In addition, conidial inoculum from an agar culture, isolated from *Holcus lanatus*, was tested. He reported successful transmission of the disease from each of these conidial sources to each of the hosts tested, from which he concluded, therefore, that there were no specialized races of *R. secalis*. Brooks (*2*), in England, has stated that *R. secalis* on "wild grasses" "undoubtedly may be a source of infection to crop plants." A suggestion of nonspecialization in Oregon has been given by Barss [11] in a report that scald was more severe on rye following barley in the rotation than when following other crops.

[11] See footnote 5.

The writer's observations of scald in the field lead to the conclusion that very restricted host specialization exists in the species *Rhynchosporium secalis*. The following field records provide evidence.

June 6, 1928, Madison, *Agropyron repens* in a field of severely infected rye was uninfected; *Bromus inermis* in a patch of severely infected *A. repens* was uninfected, while at a distance of about a mile *B. inermis* was severely infected; *Hordeum jubatum* was uninfected although growing intermixed with infected *B. inermis*.

June 21, 1928, Madison, wheat plots adjacent to severely infected barley plots were uninfected. (Wheat has been reported to be susceptible once in Germany and once in Washington.)

July 7, 1930, Madison, abundant *Agropyron repens* and timothy growing in a severely scald-infected barley plot were uninfected. (Timothy has been reported to be susceptible in Germany.)

There appears to be no explanation of the extreme diversity between the results of Bartels (*1*) and Heinsen (*12*) in Germany and those of the writer. Only one culture of the scald fungus from Germany has been studied by the writer. This was secured from the Central-Bureau Voor Schimmelcultures with the statement by Prof. Joha. Westerdijk that it is "one of the strains with which Bartels worked." In the writer's test this strain, designated B10, appeared to be specialized, producing infection only on cultivated barley (table 2). It differs in pathogenicity from most of the American cultures of the barley race only in producing symptoms on the host more slowly and sporulating less abundantly. However, one American culture, B71, behaved similarly. In culture the Bartels strain failed to produce the characteristic black pigment in old cultures as do those from North America.

The only apparent difference in the technique used in the cross-inoculation tests of Bartels and those of the writer is that the former worked mainly with host-borne conidia while the latter mainly used conidia from culture. However, in one case, Bartels reported working with inoculum from a culture isolated from *Holcus lanatus*, and finding the plurivorous relation as with host conidia from other species. On the other hand, the writer, in one inoculation trial each with the *Hordeum jubatum* and barley races, used host-borne conidia and found the same specialized condition as determined with inoculum from culture. Likewise inoculum from overwintered, naturally infected barley failed to attack rye, although it heavily infected barley. Thus the source of inoculum, i. e., from culture or host plant, did not affect the results of either Bartels or of the writer and will not serve as an explanation of their diverse results. Only the further exchange of cultures can clear up the apparently anomalous situation of extreme host specialization of a species in North America and the absence of specialization in Europe.

LIFE HISTORY

Seasonal Cycle of Occurrence

Scald becomes evident in early spring on cereals and grasses in the North Central States as soon as the first green foliage appears. With favorable conditions for development, the disease becomes progressively more prevalent on barley and rye until ripening of the crop. In the fall, volunteer barley plants may be heavily infected. The same general cycle occurs on the grasses, the disease developing abundantly in spring and fall and but little during the high temperatures of July and August.

Overwintering

The means of overwintering of *Rhynchosporium secalis* in the Northern States has not been known. It has been stated (*1*) that the organism passes the winter in an active state upon winter grains, which might account for the overwintering of the rye race and races on perennial grasses but not that of the barley race in the spring barley region. Heinsen (*12*) reported the scald fungus to be capable of saprophytic development in the soil in the greenhouse wherein its viability was maintained for a 15-month period, after which seedlings grown in the infested soil developed the disease. Bartels (*1*) also found the organism to grow in soil and persist there for a period of 6 months. The writer has been unable to detect growth of the organism on greenhouse compost soil when inoculated with a suspension of conidia and mycelium of the barley race or to secure infection of barley grown in such soil.

The possibility of overwintering and spread of scald in or on the seed was investigated in 1931 by an extensive sowing of barley seed from a severely infected field, upon soil where no scald was known to have occurred. The seed was of Wisconsin Pedigree 6 barley, produced at Madison in 1930 on severely scald-infected plants. The planting was made at La Fayette, Ind., in April 1931. No scald appeared in this planting.

Definite evidence of overwintering of *Rhynchosporium secalis* upon dead volunteer barley plants in the field has been secured by the writer at Madison. During the winters of 1926–27 and 1927–28, examination of dead infected leaves at regular intervals failed to demonstrate the survival of conidia until spring. During April and May 1927, however, the exposure of young barley seedlings as they emerged from the soil to such infected barley plant refuse resulted in heavy infection (table 4). The seedlings were kept in a high-humidity chamber and frequently sprayed with water during this exposure to the plant refuse. Thus, abundant viable inoculum was present on the overwintered plant refuse even though no conidia could be found previous to the inoculation trial.

In April 1929, dead, scald-infected barley plants that had been kept in the open field during the winter were found to bear abundant conidia after being exposed to room temperature and high atmospheric humidity for a few days. It appears probable that overwintering is accomplished by the fertile stroma of the scald fungus which resumes the production of conidia when warm temperatures return in the spring.

The observations and culture studies of other workers and the writer have failed to demonstrate the existence of a perfect stage which might serve as a spring source of inoculum.

SUMMARY AND CONCLUSIONS

Scald caused by *Rhynchosporium* spp. attacks primarily the leaf blades and to a lesser extent the leaf sheaths of barley, rye, and a number of grasses in the United States. The symptoms of the disease are the appearance of darkened water-soaked leaf spots, usually of lenticular shape followed by a drying and bleaching of the lesions which develop typical dark-brown margins. The simultaneous occurrence of many water-soaked areas covered by a gray subcuticular mycelium produces the appearance of scalding.

Scald is widespread in Europe and North America and has been reported from Tunis, Argentina, Peru, and Australia. The disease is often of little economic importance but is severe in the Pacific Coast States where estimated losses have amounted to from 20 to 30 percent.

An emended description of the genus *Rhynchosporium* has been presented, based upon the distinctive fertile stroma and manner of sporulation of the type species, *R. secalis* (Oud.) Davis. The valid binomial of the scald fungus occurring on barley, rye, and a number of grasses is *R. secalis* (Oud.) Davis.

Scald of *Dactylis glomerata* L. is caused by a fungus species that is definitely a member of the genus *Rhynchosporium* but has cylindrical conidia, which differentiate it from *R. secalis*, which has apically beaked conidia. The new species on *D. glomerata* has been described here as *R. orthosporum* sp. nov.

At present *Rhynchosporium secalis* and *R. orthosporum* are the only legitimate species in the genus *Rhynchosporium* as here defined. *R. alismatis* (Oud.) Davis, occurring on *Sagittaria* and *Alisma*, is excluded from the genus *Rhynchosporium* since its conidia are borne on flask-shaped conidiophores and it lacks the characteristic fertile stroma of this genus.

A high degree of host specialization has been found within the species, *Rhynchosporium secalis*. Six specialized races were found which can be distinguished by their capacity or incapacity to parasitize the host plants, namely, *Secale cereale* (rye), *Hordeum vulgare* (barley), *Agropyron repens*, *Bromus inermis*, *Elymus canadensis*, and *H. jubatum* in greenhouse and field inoculations. The existence of specialized races also is evidenced in the natural occurrence of this fungus in the field. Each race has displayed some constant and distinctive characters in artificial culture.

The conidia of the different races of *Rhynchosporium secalis* produced upon the hosts showed no important morphologic differences, while in culture distinct and constant differences between certain races were evident both in form of conidia and cultural characters.

Conidial germination proceeds readily in distilled water, occurring within the temperature range 4° to 28° C. The optimum temperature for germ-tube elongation lies between 18° and 21°. In distilled water, at and above a temperature of 30°, the conidia rupture and extrude the cytoplasm into the surrounding medium.

Fructification of the fungus is inhibited at the low relative humidities afforded by the greenhouse in winter, but occurs abundantly at high humidities.

Parasitism of barley by *Rhynchosporium secalis* is initiated by direct penetration of the leaf cuticle from appressoria, the mycelium establishing itself and making its initial development in the subcuticular position.

The enlargement of the infected area is accomplished by spread of the subcuticular mycelium. The epidermal cells collapse under the subcuticular mycelium being the first tissue to show the effects of the disease. Following epidermal collapse, the mycelium repeatedly penetrates from the subcuticular position into an intercellular position within the mesophyll, causing almost immediate collapse of that tissue. Relatively sparse development of mycelium occurs in the mesophyll tissue.

The subcuticular mycelium proliferates and enlarges, pushing the cuticle away from the epidermal wall, to form a fertile stroma, from

one to several cells in thickness, on the lesion surface. Fructification follows on the fertile stroma, covering the collapsed area of the lesion. Conidia are borne directly on the sides of the cells of fertile stroma on the surface of the lesion.

The barley race has been shown to overwinter in Wisconsin on dead tissues of plants infected during the previous season.

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THE BIOLOGY OF PLATYGASTER HERRICKII, A PARASITE OF THE HESSIAN FLY [1]

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INTRODUCTION

Platygaster herrickii Packard [3] is an important parasite of the hessian fly, *Phytophaga destructor* (Say), in certain sections of the United States. Little has been published on it except from the taxonomic point of view. In 1933 Gahan (*1*) [4] gave a history of the literature together with a full redescription of the adult. Valuable unpublished observations on its behavior and development were made as early as 1916, by the late W. R. McConnell, and descriptions of egg, first-instar larva, and methods of oviposition were deposited by him in official files of that year. The senior author began observations of its morphology and activity in the eastern coastal States in 1920, and since then has accumulated information as he has had the opportunity. More recently the junior author began work on the occurrence and habits of this parasite in the West Central States.

DISTRIBUTION AND ECONOMIC IMPORTANCE

Platygaster herrickii has been collected from most of the winter-wheat-growing sections of the United States (fig. 1). It is an important parasite of the first spring brood of the hessian fly in the southeastern portion of the wheat-growing sections and west of the Mississippi River in parts of Missouri, Iowa, Kansas, Nebraska, and Oregon. Parasitization by this species has been found to be as high as 25 percent of the host puparia in North Carolina, 20 percent in the extreme southern part of Virginia, and 3 percent in central Virginia. North of latitude 38° in the eastern coastal States little parasitization has been observed. Surveys made in 1931 and 1932 showed 36 percent parasitization by this species in southwestern Iowa, 14 percent in Missouri, 6 percent in Nebraska, and 1 percent in northeastern Oklahoma. In Oregon, Rockwood (*10*) has reported *P. herrickii* as parasitizing large numbers of hessian flies, but he says that it appears to be more susceptible to desiccation than either its host or the serphoid parasite *P. hiemalis* Forbes. Reeher found, in the vicinity of Forest Grove, Oreg., in 1930 an average of 14 percent parasitization by this species, with a maximum of 33 percent in one field, and in 1931 an average of more than 16 percent.

In the regions of its greatest abundance *Platygaster herrickii* usually occupies more species of hosts than do any of the other hessian

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[2] The authors acknowledge their indebtedness to P. N. Annand, under whose general supervision the work was done; to M. M. Reeher for contributions on the biology of the parasite in the State of Oregon; to C. M. Packard and W. R. Walton for criticism of the manuscript; to J. R. Horton for helpful suggestions; and to H. D. Smith, J. S. Pinckney, H. H. Walkden, and E. T. Jones for various collections of material and distributional records.
[3] Order Hymenoptera, superfamily Serphoidea.
[4] Reference is made by number (italic) to Literature Cited, p. 213.

fly parasites except *Eupelmus allynii* (French), and, in terms defined by Smith (*12*), it is "extrinsically superior" to them. However, as it is practically defenseless against the typical chalcidoid parasites when competing with them in the same host, it may be considered as "intrinsically inferior" to all the principal chalcidoids.

As a supplementary parasite *Platygaster herrickii* is important largely for two reasons: (1) It works abundantly in those sections of the South and West where another important serphoid parasite of the hessian fly, *P. zosine* Walker, fails to thrive, and thus helps to maintain a higher degree of parasitization than would otherwise be possible; and (2) by ovipositing in the egg instead of the later stages of the host, as do the chalcidoids, it is able to parasitize hosts that later in the season become inaccessible to chalcidoid attack.

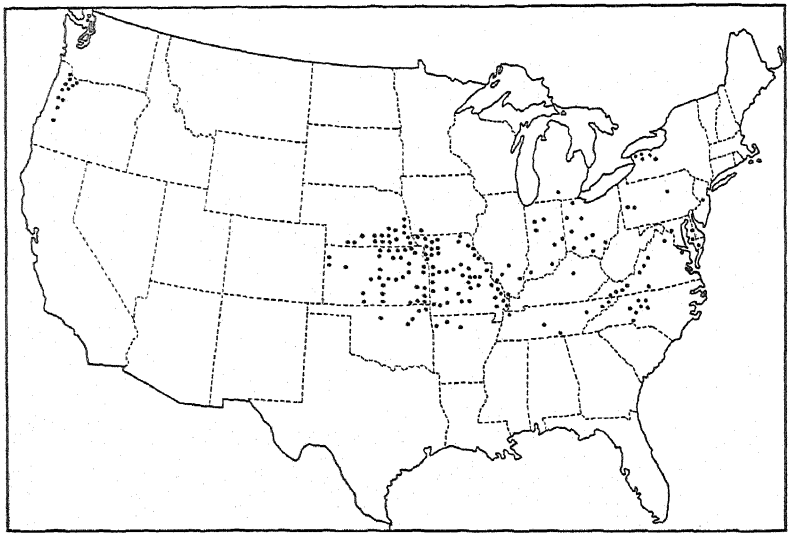


FIGURE 1.—Geographical distribution of *Platygaster herrickii*. Dots show localities where specimens have been collected.

TECHNIQUE

Specimens were studied in toto while alive in normal salt solution or Ringer's solution and also after having been killed and fixed in Bouin's fluid. Parasitized host larvae for sectioning were fixed in Bouin's fluid. Most of the parasitized host eggs, however, were fixed in Hermann's fluid or Flemming's solution, which stained them black and rendered them easier to handle in embedding and sectioning. Further to facilitate handling, several eggs at a time were tied up in tiny, fine muslin bags before being passed through the reagents prior to embedding. The paraffin method was used with a Minot rotary microtome. Iron haematoxylin was used for staining the microtomic sections illustrated in figure 5, *B* to *H*, and chloral haematoxylin counterstained with orange G for those illustrated in figure 6, *A* to *H*.

THE ADULT [5]

GENERAL APPEARANCE AND SEX DIFFERENCES

The adult (fig. 2, *A*) is approximately 1.5 to 2.2 mm long, and shiny black with grayish pubescence. The ovipositor is curved, slender, slightly attenuated, and each sheath valve (third valvula) (fig. 5, *K*) bears seven setae near its extremity and three hyaline tubercles distributed along its distal half. The dark sclerotic portion of the sheath ranges in length from 0.33 to 0.44 mm.

The sexes may be differentiated by the antennae (fig. 2, *B*), the second joint of the flagellum being broader in the male than in the

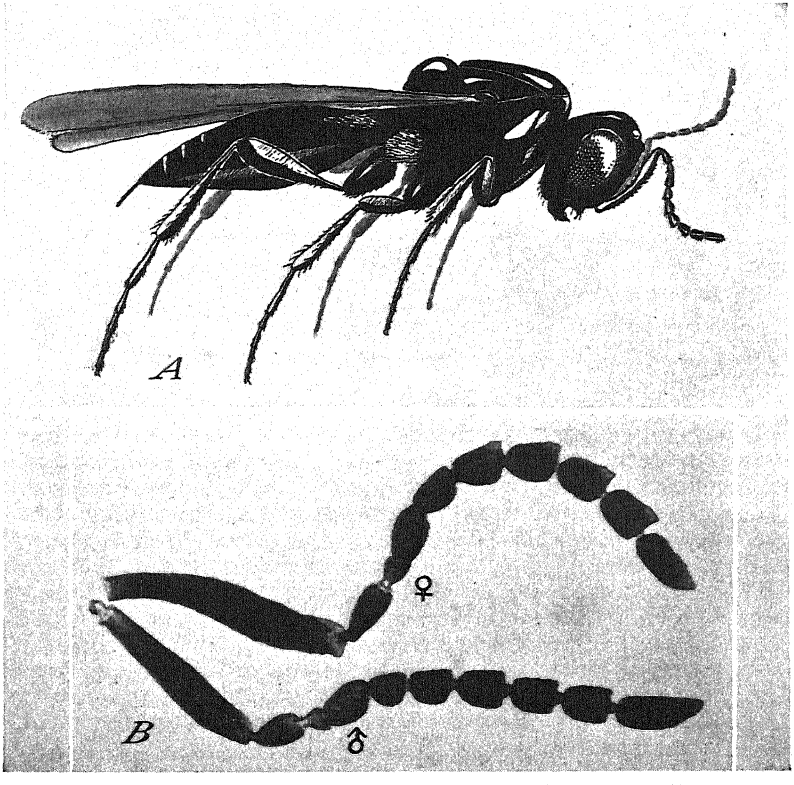


FIGURE 2.—*Platygaster herrickii: A*, Adult female, ×32; *B*, male (♂) and female (♀) antennae, ×85.

female, and by the shape of the abdomen, which is distinctly pointed in the female, whereas in the male it is more spatulate and the tip curves more definitely downward. The wings protrude farther beyond the tip of the abdomen in the male than in the female.

COMPARISON WITH OTHER SERPHOID PARASITES OF THE HESSIAN FLY

Platygaster herrickii can be distinguished from the other serphoid parasites of the hessian fly, *P. zosine* and *P. hiemalis*, by its distinctly larger size and the presence of conspicuous parapsidal grooves. It is

[5] For a technical description of the adult refer to Gahan's (*1*) redescription published in 1933.

about the same size as and in many respects resembles both *Trichacis remulus* (Walker) and *Platygaster pleuron* (Walker). It may readily be distinguished from *T. remulus* by the absence of the tuft of short gray hairs near the apex of the scutellum which characterizes the latter parasite, and it differs distinctly from *P. pleuron* by the absence of the many longitudinal striations along the basal half of the dorsal and ventral surfaces of the abdomen (fig. 3). The face of *P. herrickii* is so faintly punctate as to appear smooth and shiny except for coarse rugulations near the base of the antennae, while the face of *P. pleuron* is closely and distinctly punctate and has no conspicuous rugulations.

The ovipositor also distinguishes *Platygaster herrickii* from these other serphoids, although there is a marked resemblance in all except *P. zosine*. As in the case of *P. herrickii*, each ovipositor sheath valve of both *P. hiemalis* and *Trichacis remulus* bears only three of the hyaline tubercles, but the entire ovipositor of *P. hiemalis* is only about one-third as large as that of *P. herrickii*, and in *T. remulus* the tubercles are placed much closer together. In *P. pleuron* the ovipositor sheath valve bears five or six hyaline tubercles instead of three, a distinct point of differentiation. In *P. zosine* the ovipositor is a third smaller, with sheath valves straight instead of curved, and blunt at the extremity instead of attenuated.

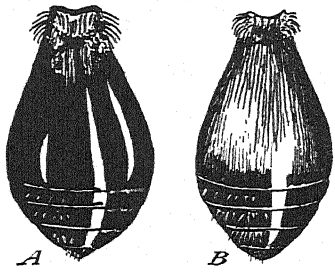


FIGURE 3.—Dorsal surface of the abdomen of (*A*) *Platygaster herrickii* and (*B*) *P. pleuron*. ×37.

OVIPOSITION

The female oviposits in the hessian fly egg. The host eggs are slender, reddish, rod-shaped, about 0.4 mm long, and are usually found sparsely scattered over the upper surface of the wheat leaves.

In searching for the eggs, the parasite walks hurriedly over the wheat leaves and constantly vibrates her antennae, letting their tips brush the surface of the leaf. Upon striking a hessian fly egg she strokes it rapidly with the tips of both antennae for about half a second. Then, with body parallel to the length of the egg, she draws herself over and a short distance beyond it and feels about for it with the tip of her extended ovipositor. Sometimes she misses the egg entirely and walks away, but more often she finds it and inserts the ovipositor into its upper surface. While depositing an egg she stands with head and antennae down, wings folded over the back, and motionless except for a slight rhythmic movement of the abdomen. She appears to oviposit quite as readily when facing downward as when facing upward on the leaf.

Two females were timed for the number of seconds the ovipositor was held in a host egg. In 30 ovipositions the time was found to range from 5 to 20 seconds, with an average of 14.

Host eggs freshly oviposited into by *Platygaster herrickii* showed only a single egg per insertion, and observations made by the late W. R. McConnell agree with this.

HABITS AND OTHER CHARACTERISTICS

When emerging from the host, the parasite makes a circular or irregular hole from 0.5 to 0.7 mm in diameter near one end of the puparium (fig. 4).

An examination of 200 *Platygaster herrickii* adults, taken at random from eight localities in Missouri, three in Nebraska, and one in Kansas, showed 102 females and 98 males, which indicates the sex ratio to be nearly equal.

Platygaster herrickii is capable of parthenogenetic reproduction, and, as is well known, this results in male offspring only.

The adult *Platygaster herrickii*, when molested, appears to feign death, drawing its legs and antennae up close to the body, although in actual death the antennae and legs are stretched away from the body. If disturbed while crawling over the wheat leaves, it quickly drops to the ground. It is positively phototropic, as demonstrated by its behavior under the writers' observation for many years.

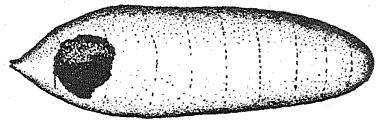


FIGURE 4.—Hessian fly puparium showing exit hole through which an adult *Platygaster herrickii* has escaped. × 22.

THE EGG AND EMBRYONIC DEVELOPMENT

DESCRIPTION OF EGG AT TIME OF OVIPOSITION

The egg (fig. 5, *A*) of *Platygaster herrickii* is hyaline, highly refractive, and immediately after oviposition is 0.06 mm long and 0.03 mm wide. It is subellipsoidal, with the caudal extremity slightly attenuated and usually turned a little to one side. The cephalic end is well rounded, with two or three short, flagellumlike, follicular adherencies.

EMBRYOLOGICAL DEVELOPMENT

The parasite egg develops simultaneously with that of its host. A sagittal section of a host egg with a freshly deposited parasite egg lying within it is shown in figure 6, *C*. Figure 5, *B*, depicts the same parasite egg greatly enlarged and shows the position of sperm and nucleus. An egg 3 hours old, showing the appearance at first maturation, is illustrated in figure 5, *C*, and eggs 3 days old, which have reached the four-cell cleavage stage, in figure 5, *D* and *E*. The development so far described is essentially like that of *Platygaster hiemalis* Forbes and *P. vernalis* (Myers) (=*P. zosine* Walker) as related by Leiby and Hill (*5*, *6*), except that at the time the embryonic nuclei (*em*) reach the four-cell cleavage stage the paranuclear mass (*pa*) within the trophamnion (*tro*) has not yet broken up into the several small distinct masses such as are usually found in *P. zosine*.

The embryo continues to develop in monembryonic manner, rather than by polyembryony as in the case of *Platygaster zosine* or by twinning as does *P. hiemalis*. Successive advancements of development in the morula and blastula forms are shown in figure 5, *F*, *G*, and *H*, and figure 6, *A*. These stages are similar to those shown by Silvestri (*11*) for *P. dryomyiae* Silv., and to those of the monembryonic forms of *P. hiemalis* as shown by Leiby and Hill (*5*). *P. herrickii*, however, does not accumulate about itself an adventitious cyst of host tissue as do these other species of *Platygaster*.

PSEUDOGERMS

Beginning with the blastula stage, an interesting demarcation of development takes place in the paranuclear body of the trophamnion. During the period of blastula formation this body becomes broken

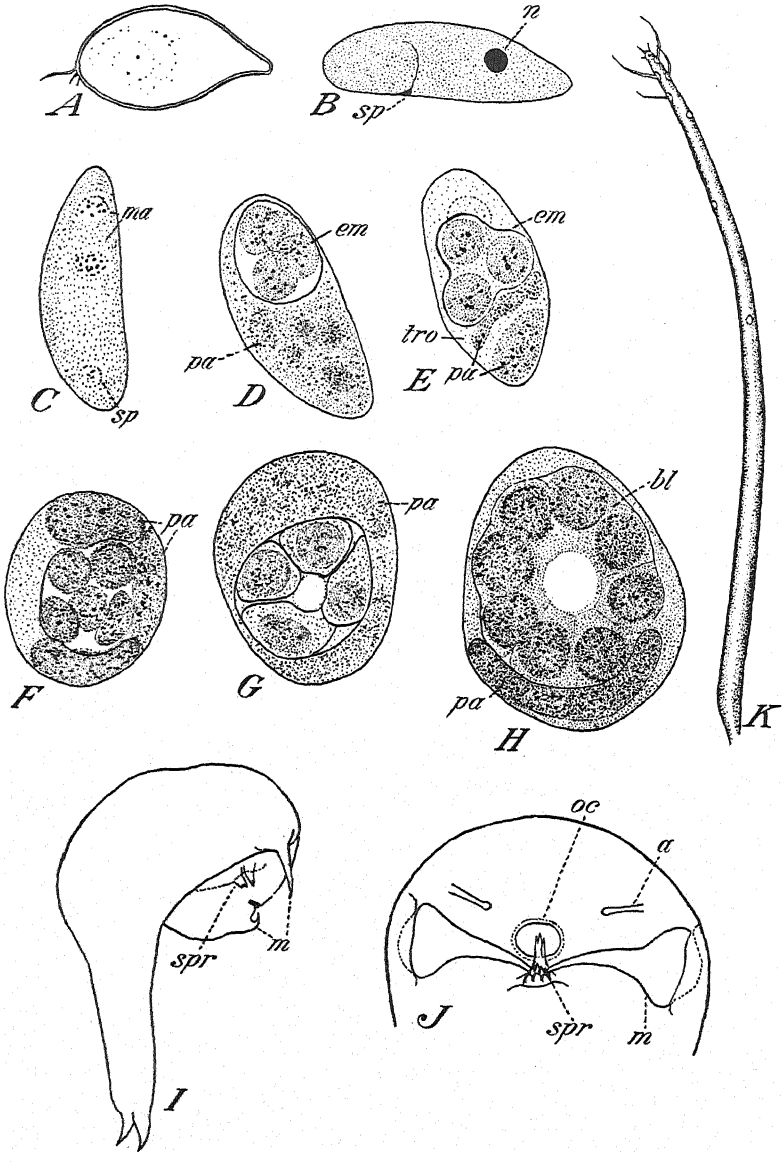


FIGURE 5.—*Platygaster herrickii: A*, Freshly deposited egg, × 433; *B*, sagittal section of freshly deposited egg with nucleus (*n*) and sperm (*sp*), × 1,306; *C*, egg 3 hours old showing sperm or male nucleus (*sp*) and first maturation stage (*ma*), × 1,050; *D* and *E*, eggs 3 days old showing embryo (*em*) at the four-cell stage of division, paranuclear mass (*pa*), and trophamnion (*tro*), × 970; *F*, section of egg 2 days old in morula stage, with paranuclear mass (*pa*) beginning to break up, × 1,087; *G*, section of egg 4 days old beginning to show blastula characteristics, × 1,000; *H*, section of another egg 4 days old showing distinct blastula formation (*bl*) and a well-defined paranuclear mass (*pa*), × 974; *I*, dorsal aspect of primary larva with head bent forward in normal position, showing mandibles (*m*) and sclerotic projection (*spr*) on inferior lip, × 273; *J*, ventral aspect of head, showing antennae (*a*), oral cavity (*oc*), mandibles (*m*), and sclerotic projection (*spr*) on inferior lip, × 314; *K*, sheath valve of ovipositor, × 270.

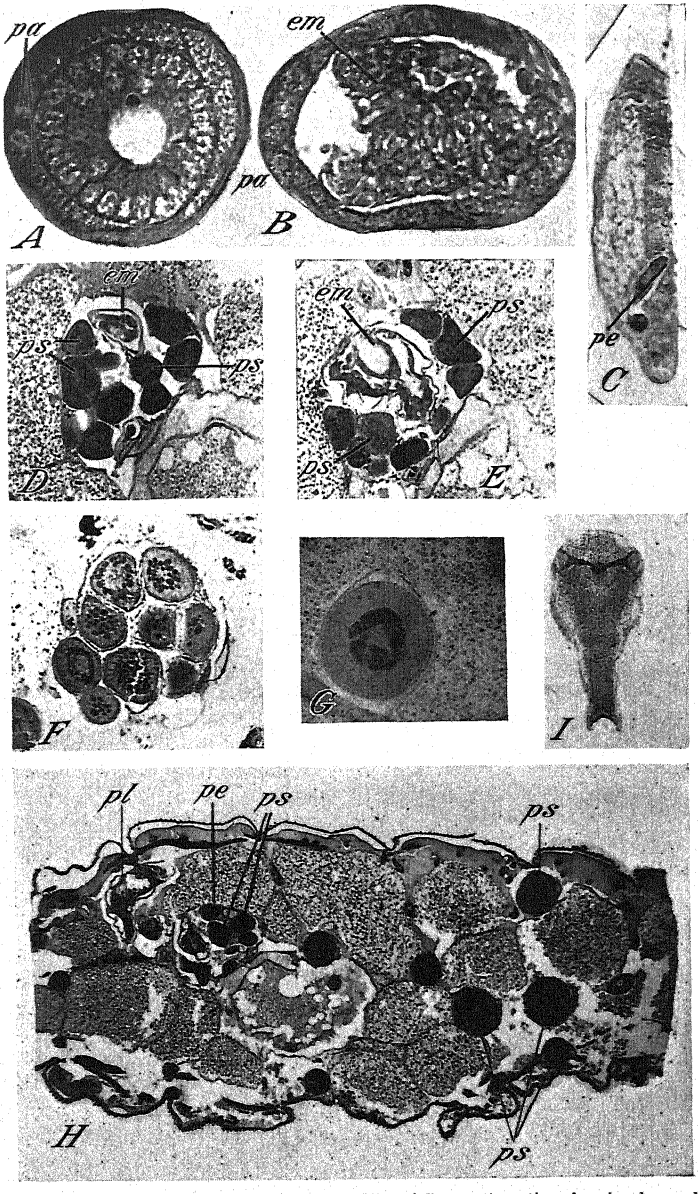


FIGURE 6.—Photographic studies [6] of *Platygaster herrickii: A*, Microtomic section of egg in advanced blastula stage, showing paranuclear mass (*pa*) breaking up into several bodies, × 425; *B*, section of egg showing embryo (*em*) in advanced stage of development and paranuclear mass (*pa*) divided into numerous small bodies, × 350; *C*, sagittal section of a host egg with a freshly deposited egg (*pe*) lodged within, ×1241; *D* and *E*, sections at different points through a parasitic body with embryo (*em*) about ready to hatch and pseudogerms (*ps*) which have commenced precocious development, × 116; *F*, section through a cluster of pseudogerms shortly after the larva has hatched, × 106; *G*, cross section through a single pseudogerm, × 131; *H*, sagittal section through a portion of a hessian fly larva showing parasite egg (*pe*) in an advanced stage of development with pseudogerms greatly enlarged, a primary larva (*pl*) that has recently hatched from another egg, and pseudogerms (*ps*) released from this egg and scattered about in the body cavity of the host, × 55; *I*, ventral aspect of entire primary larva. × 94.

[6] Photomicrographs made by E. J. Udine.

up (fig. 6, *A*), and as the embryonic development progresses many distinct bodies appear (fig. 6, *B*). In the individual illustrated (fig. 6, *B*) the embryonic membranes are forming and the paranuclear mass has already divided into many nuclei. Some of the best defined ones are oval and 0.016 mm long. At this stage the paranuclear bodies begin a precocious development, and since they superficially imitate the development of true germs the writers will call them "pseudogerms", as Marchal (*8, p. 600*) has called similar bodies in other species of *Platygaster*.

Two sections through a parasite larva fully formed and about ready to hatch are shown in figure 6, *D* and *E*. It may be observed that the paranuclear bodies have increased considerably in size. Measurements showed them to range from 0.03 to 0.06 mm in length. Moreover, nucleated masses of chromatin have become distinctly visible in each body. These bodies continue to grow after the egg has hatched, and the granules of chromatin become large and either grouped in the center of the body or arranged in blastulalike formation. Figure 6, *F*, shows a section through a group of pseudogerms that are still clinging together within a partly ruptured chorion through which the larva has broken in the process of hatching. The pseudogerms in this group were larger than those shown in the earlier embryonic development, measuring from 0.057 to 0.086 mm in diameter. In this case the newly hatched larva was lodged nearby in the body cavity of the host. The pseudogerms finally break loose entirely and become distributed throughout the body cavity, as shown in figure 6, *H*. At this stage they are spherical and protected by a chorionlike wall. They continue to increase in size while loose in the body cavity of the host. Measurements of 176 pseudogerms taken at random from numerous hosts under natural field conditions and examined in physiological media showed diameters ranging from 0.04 to 0.33 mm, with an average of 0.14 mm. Figure 6, *G*, is a section through the center of a typical pseudogerm that measured 0.13 mm in diameter after fixation. This section shows the disposition of chromatin particles and the chorionlike protecting wall.

No true embryonic development of a pseudogerm was ever observed, and examinations, later in the season, of parasitized hessian fly puparia from the field showed the pseudogerms in the process of disintegration, and eventually consumed by the parasite larva.

Out of eight flies parasitized in the field in which there was but one first-instar larva, the number of pseudogerms was found to range from 12 to 38, with an average of 25. In 54 cases in which more than one larva was found in a single host, the average was 22 pseudogerms per embryo. The maximum number found in any host was 145, and judging from the larvae present in this host, these were the product of six eggs.

Figure 6, *H*, shows a sagittal section of part of a hessian fly larva containing in its body cavity a parasite egg (*pe*) with the embryo about ready to hatch, and pseudogerms (*ps*) still retained within the body walls of the parasite. Nearby is a primary larva (*pl*) that has recently hatched and released a number of pseudogerms (*ps*) which have grown and become distributed throughout the body cavity.

The development of pseudogerms of this character was first discovered by Marchal (*8*), who found them occurring in *Platygaster lineatus* Kief., *P. marchali* Kief., and *Trichacis remulus*. In all these

species the pseudogerms, like those of *P. herrickii*, represent precociously developed paranuclear bodies of the trophamnion and are released into the body cavity of the host when the primary larva liberates itself from the embryonic mass. In these species they differ, however, from those of *P. herrickii* in that after liberation they not only grow but multiply.

The pseudogerms described by Parker (*9*) for the braconid parasite *Macrocentrus gifuensis* Ashmead differ somewhat from those found in conjunction with the species of *Platygaster*. With *M. gifuensis*, which is polyembryonic, the pseudogerms are released into the body cavity of the host during the development and dissociation of the morulae, instead of at the end of embryological development, and they are usually associated with parts of the trophamnion instead of being confined to the paranuclear masses.

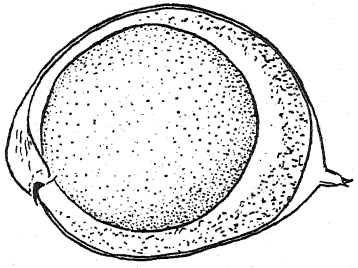


FIGURE 7.—Lateral aspect of primary larva of *Platygaster herrickii* in an advanced stage of growth. × 135.

The occurrence of these pseudogerms affords a valuable aid in identifying *P. herrickii* in its early stages, for, with the exception of *Trichacis remulus*,[7] this is the only hessian fly parasite in the United States known to produce such bodies. *T. remulus*, however, may be identified by the primary larvae, which offer marked distinguishing characteristics.

THE PRIMARY LARVA

The young larva (fig. 5, *I* and *J*, and fig. 6, *I*) is colorless and translucent. A typical specimen measured 0.288 mm in length when extended and 0.130 mm across its greatest width. It is cyclopean in form, with the cephalic region slightly longer and considerably wider than the caudal region. The caudal extremity is bifurcated with two pointed, backward-curving hooks. Two-thirds of the way forward on the abdomen there is a well-defined joint at which the caudal region may be bent forward under the head. The mandible is 0.036 mm long and curved near the extremity. The buccal opening is circular, and the inferior lip bears two small, closely packed groups of tiny sclerotic projections, one group at the edge of the buccal opening and the other on a short, elevated sclerotic ridge just caudad of the first group. These projections vary in number and size. In one specimen examined there were four such in the first group and nine in the second group; in another, three and seven, respectively. The extremities of the mandibles, when brought together, rest between the two groups. Two slender, slightly knobbed antennae, 0.013 mm long, are present a short distance in front of the mandibles.

In its advanced development (fig. 7) the primary larva is almost spherical and several times its original size. The caudal appendage becomes functionless and eventually collapses.

Frequently two or more first-instar larvae are found in a host, but only rarely does more than one reach maturity. In a few instances two adults have been reared from the same host and in one case four pupae were found in one host.

[7] *Trichacis remulus* was introduced into the United States from France in 1935.

The primary larva of *Platygaster herrickii* can readily be distinguished from those of *P. zosine* and *P. hiemalis* because neither of these species is cyclopean in form. *Trichacis remulus*, as described by Marchal (*7, 8*), is conspicuously different from *P. herrickii* in having footlike processes at the base of the head, and in the shape of the antennae. The senior writer, however, noted a striking resemblance to *P. pleuron*, although there seemed to be a slightly different arrangement in the sclerotic projections on the inferior lip.

References to the larval form of *Platygaster herrickii* were made by Kulagin (*4*) in 1898 and by Hill (*3*) in 1926. Kulagin, however, was incorrectly identifying an entirely different species, as has been pointed out by both Marchal (*8, p. 490*) and Hill (*3*).

THE MATURE LARVA

The mature larva (fig. 8) is white, shining, and somewhat reniform. A typical specimen was 2.1 mm long and 0.9 mm wide. There are 11

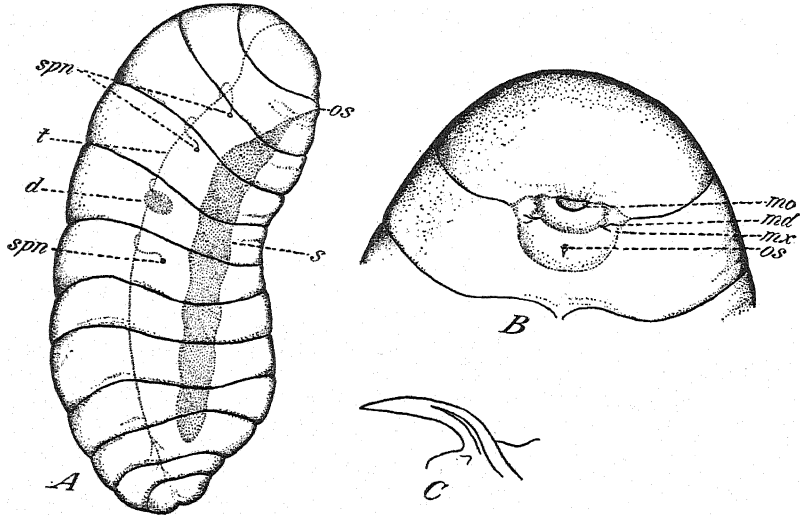


FIGURE 8.—Mature larva of *Platygaster herrickii*: *A*, Lateral aspect showing segmentation of body, spiracles (*spn*), lateral branch of tracheal system (*t*), discoidal body (*d*), lateral branch of silk gland (*s*), and silk-gland opening (*os*), × 33; *B*, head and part of thorax showing mouth (*mo*), mandibles (*md*), sclerotic ridge on maxillary area (*mx*), and silk-gland opening (*os*), × 77; *C*, mandible, × 450.

well-defined body segments, excluding the head. Spiracles are present on the second and third thoracic segments and the second abdominal segment. On each side of the first abdominal segment there is a large discoidal body[8] (fig. 8, *A*, *d*) under the cuticle at the terminus of the lateral tracheal branch. The buccal opening (fig. 8, *B*, *mo*) is a simple crescent-shaped orifice capable of being opened or closed by the soft superior lip controlled by radiating muscles. The mandibles (fig. 8, *B* and *C*) are 0.0466 mm long, slightly curved, and darkly sclerotic. They occur somewhat posterior to the mouth and are widely separated. In one specimen their bases were 0.2 mm apart. Each of the maxillary areas bears a sclerotic ridge (fig. 8, *B*, *mx*) immediately posterior to the mandibles. The silk-gland opening

[8] This body is characteristic of many species of *Platygaster* and has been described by Hill (*2, 3*) as occurring in *P. zosine* (*P. vernalis*) and *P. hiemalis*, by Marchal (*8*) in *P. ornatus*, and by Silvestri (*11*) in *P. dryomyiae*. Its function is obscure, although Silvestri calls it a gland.

(fig. 8, *A* and *B*, *os*) lies on the median line posterior to the mandibles. The silk glands (fig. 8, *A*, *s*) are two single tubes, one extending along each side of the body as far as midway of the sixth abdominal segment.

During this stage most of the host contents are consumed, but not until the host has formed its dark puparium.

The mature larva resembles those of the other serphoid parasites of the hessian fly, although the mandible of a specimen of *Platygaster pleuron* was found to be slightly longer than that of *P. herrickii*. According to Marchal (*7*), the mandibles of *Trichacis remulus* are sharply curved and borne on two large tubercles, which is not the case in *P. herrickii*. The mature larva of *P. herrickii* is about twice as long as that of *P. zosine* and the mandibles are longer by 0.016 mm. This may seem a small difference, but in both species the dimensions of the mandibles have been found to be constant. The larval body of

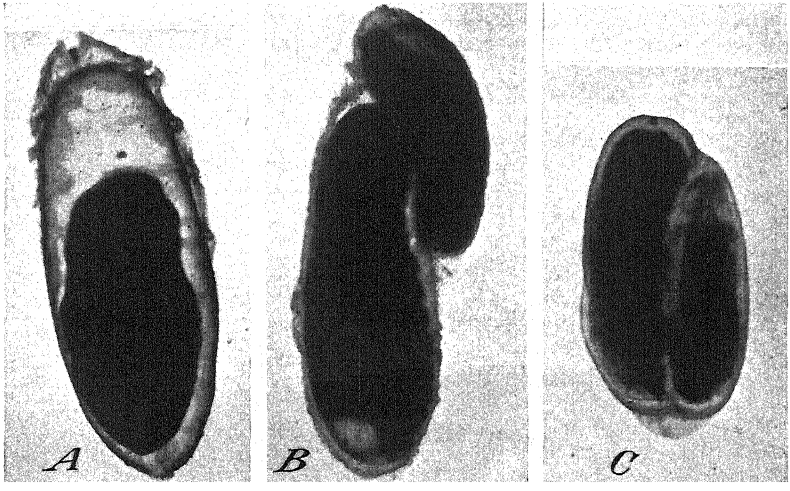


FIGURE 9.—Photomicrographs of *Platygaster herrickii*: *A*, Cocoon with larval skin of host adhering to the outside; *B* and *C*, examples of two cocoons found in a single host, greatly enlarged.

P. herrickii is also about twice as large as that of *P. hiemalis*, with mandibles slightly longer and distinctly more curved.

THE COCOON

The cocoon of *Platygaster herrickii* is made of straw-colored, thin, shiny, tough, elastic material. It is ovoid in shape and varies somewhat in size. Ten specimens ranged from 2.17 by 0.87 mm to 3.22 by 1.31 mm, with an average of 2.87 by 1.12 mm. The cocoon usually occurs singly within the host puparium, which it nearly fills, but two and even three cocoons have been found in one host. Figure 9 shows (*A*) a cocoon removed from the host puparium but still within the host skin and (*B* and *C*) two cocoons in one host.

THE PUPA

The pupa (fig. 10) is white when first formed, as in other Hymenoptera, but it soon darkens. Beginning with the compound eyes, the color spreads until finally the entire body, except the thin integument between the abdominal plates, becomes shiny black.

SUPERPARASITISM

Superparasitism appears to be common with this species. Females in the laboratory cages oviposited into eggs that had already been parasitized by other females. They also oviposited repeatedly into the same egg, three or four ovipositions being observed frequently. Out of 20 parasitized hessian flies taken from a field near Salisbury, N. C., 12 contained more than one larva per host, distributed as follows:

Larvae	*Occurrences*	*Larvae*	*Occurrences*
1	8	4	2
2	3	6	2
3	4	8	1

The occurrence of more than one larva per host apparently is not due to polyembryony, for no evidence of polyembryony was found in the embryological studies. Furthermore, in many hundreds of rearings only rarely did more than one adult develop in a host.

SEASONAL HISTORY AND MORTALITY

Platygaster herrickii has one generation a year. The adults emerge in the spring simultaneously with the hessian flies and oviposit into

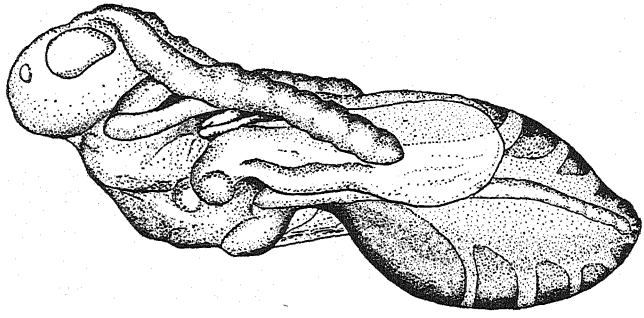


FIGURE 10.—Pupal stage of *Platygaster herrickii* before it has hardened. × 35

their eggs. The parasite eggs hatch and reach the primary larval stage in the course of a few weeks, and by the end of the summer the larvae are full grown. The cocoon is soon formed, pupation takes place, and the adult stage is attained before winter. The adults hibernate within the cocoons inside the host puparium until warm spring weather induces emergence.

Material from Abington, Va., examined on June 30 contained only embryos, but by July 12 primary larvae were found, and on July 15 one mature larva. Other material collected at the same locality on August 17 showed *Platygaster herrickii* predominant in the primary larval stage, although again one full-grown larva was discovered. By August 24 primary and mature larvae and white pupae were found, and by September 9 white pupae and one fully developed adult were present within their cocoons.

In the vicinity of Wichita, Kans., *Platygaster herrickii* was found ovipositing throughout most of April. Mature larvae appeared the latter part of July, and cocoons were formed in August. White pupae

were found by September 1, which became black by September 14 and transformed to unemerged adults by November 25. Dissections made the last of September showed parasites in all stages of development from embryos to black pupae.

Reeher says that in the vicinity of Forest Grove, Oreg., adults of this species begin to emerge, in normal years, about the middle of April, although an occasional individual may appear as early as March 26. They may be found in the fields for about a month, coincident with the normal spring emergence of the hessian fly. Examination of *P. herrickii* material taken from the fields near the

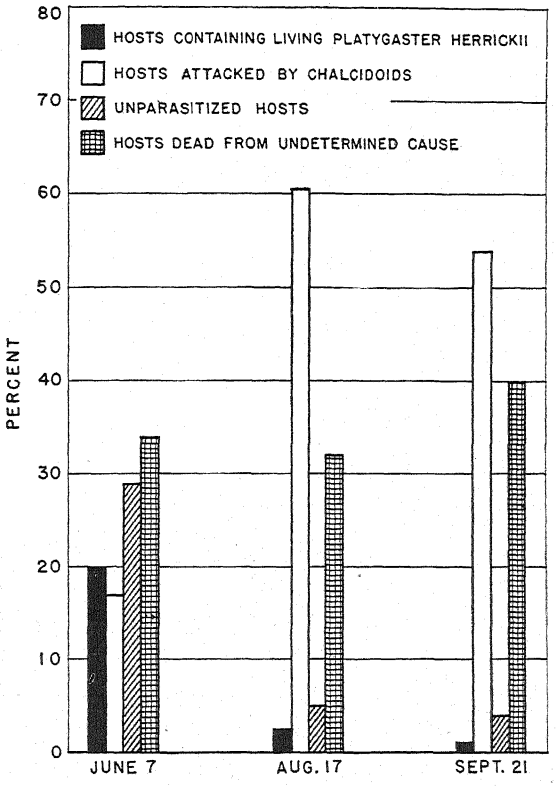


FIGURE 11.—Observations on parasitization of hessian fly puparia collected at Abingdon, Va., on three different dates in the summer of 1932, indicating mortality of *Platygaster herrickii*.

end of October showed cocoons to be already formed and usually the adult stage present within. As in the other parts of the country, hibernation normally takes place in the adult stage within the cocoon.

Platygaster herrickii parasitizes its host at the beginning of the season, before most of the other parasites have begun their work, and consequently comes into competition with all other species that attack the immature stages of the hessian fly. At a result of this multiple parasitism a high percentage of *P. herrickii* succumb during the season. This was shown by an examination of hessian fly puparia collected from a wheatfield near Abingdon, Va. Collections of 200 puparia were made on June 7, 220 on August 17, and 300 on September

21. These puparia were dissected and their contents observed. Figure 11 illustrates the increasing mortality of *P. herrickii* during the season. It will be observed that this was due chiefly to the inroads of chalcidoids and to some extent to undetermined factors. Hosts attacked by chalcidoids increased from 17 percent on June 7 to 60.5 percent on August 17. By September 21 other conditions interfered sufficiently to leave only 54 percent recognizable as attacked by chalcidoids. Those dead from undetermined causes increased from 34 percent in June to 40 percent in September. The decrease of 2 percent in August can be ignored as due to unavoidable deviations between samples. Consequently, not only were unparasitized hosts reduced from 29 percent in June to 4 percent in September, but those containing living *P. herrickii* were likewise reduced from 20 percent in June to 2.5 percent in August and to 1 percent in September, showing a total mortality of 95 percent of *P. herrickii* during the season.

The species of Chalcidoidea involved were *Eupelmus allynii*, *Merisus destructor* (Say), *Tetrastichus carinatus* Forbes, and *Merisus febriculosus* Girault. The number of the last two was insignificant, but *E. allynii* formed 10 percent of the total on June 7 and 39 percent on August 17. The proportion of *M. destructor*, which was 6 percent on the first dissection, had increased to 10 percent by August 17. Much of the mortality of *P. herrickii* in the vicinity of Abingdon may therefore be attributed to these two parasites.

Since most of the chalcidoid activity took place prior to August 17, collections in this locality should be made early in the season to obtain the maximum number of *Platygaster herrickii*.

In Kansas conditions were more favorable for avoidance of multiple parasitism, as most of the hosts parasitized by this species occurred on the small, nonproductive culms beneath the surface of the soil where they were not easily accessible to puparial parasites.

SUMMARY

Platygaster herrickii occurs as a parasite of the hessian fly throughout most of the winter-wheat-growing sections of the United States. It is an important parasite in the more southern parts of the eastern wheat-growing sections, in the central wheat belt west of the Mississippi, and in western Oregon.

The adult is from 1.5 to 2.2 mm long and is black and shining.

The egg is deposited in the egg of the host, where it develops simultaneously with that of the host. It is hyaline, subellipsoidal, and about 0.06 mm long at the time of deposition. Its development takes place monembryonically, and at the time of hatching numerous spherical pseudogerms are released into the body cavity of the host, where they become generally distributed.

The primary larva, at time of hatching, is about 0.29 mm long, of cyclopean type, and has a bifurcate caudal extremity. Later in its development it becomes swollen and nearly spherical. The mature larva is white, shining, about 2 mm long, segmented, and reniform. While in this stage it consumes the host contents, but not before the dark puparium of the hessian fly has been formed.

The cocoon is formed within the puparium of the host. It is ovoid and made of thin, elastic, straw-colored material. It is about 2.5 mm long and 1.1 mm wide.

There is one generation a year. The adult hibernates within the cocoon and emerges in the spring at the time the hessian flies are ovipositing. Embryological and larval development proceeds with the development of the host until the end of summer, when the cocoon is formed and pupation is begun. The adult stage is reached before winter.

In Abingdon, Va., heavy mortality has been found to take place in the course of the season, owing chiefly to multiple parasitism.

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OBSERVATIONS ON THE BIOLOGY OF THE WHEAT-STEM MAGGOT IN KANSAS[1]

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INTRODUCTION

The wheat-stem maggot, *Meromyza americana* Fitch (Diptera Chloropidae) is widely distributed through the wheat-growing area of North America. In infested fields it is not uncommon to find from 10 to 15 percent, and in some instances almost 100 percent, of the plants injured by this insect.

REVIEW OF LITERATURE

The literature on the wheat-stem maggot consists largely of short notes in annual reports and various periodicals.

Lugger (*20, Bull. 43, pp. 210–213*)[3] reported that the wheat-stem maggot had been found as early as 1821 in Pennsylvania, but the earliest record in the entomological literature is that of Fitch (*5, p. 299*), who in 1856 described the fly from material collected in New York and gave it the name *Meromyza americana*. Walsh and Riley (*24*) and Riley and Fuller (*22*) reported it as occurring in Missouri. Lintner (*18, 19*) noted its occurrence in New York and included notes on the nature of the injury, the larval and pupal forms, and the time of appearance of the fly. Webster (*25, pp. 389–390*), in Ohio, reported finding a full-grown larva in a stem of wheat on June 14; on June 16 a larva was found in the upper joint of a growing straw; on June 24 puparia were collected; and on July 18 he observed the copulation of emerged adults. He also reported the frequent occurrence of the mite *Heteropus ventricosus* Newport as a parasite of the maggot. Forbes (*9*) reported the maggot in Illinois and described the immature stages of the insect. He also collected and described the parasite *Coelinius meromyzae*. Forbes (*10*) contributed notes on the life history of the maggot and suggested the probability of a midsummer brood in self-sown grain, making a total of three broods each year. Fletcher (*6*) reported injury in both spring and fall in Canada, and the collection of a specimen of hymenopterous parasite thought to be a new species or a variety of *Coelinius meromyzae* (Forbes).

Webster (*26*) found the wheat variety Velvet Chaff to be more highly infested with the wheat-stem maggot than Michigan Wonder. Garman (*14*) reported the occurrence of the insect in Kentucky,

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[3] Reference is made by number (italic) to Literature Cited, p. 237.

where it was especially common in blue-grass pastures, even when closely grazed. Fletcher (7) stated that *Agropyron divergens* Nees (awned bluestem) was badly injured by the maggot. Osborn and Gossard (*21*) recorded the occurrence of the maggot in Iowa and reported it as being preyed upon by the parasite *Coelinius meromyzae* Forbes and by two other undetermined species of parasites. Webster (*27, pp. 74–79*) spoke of it as being possibly the most widely distributed of all wheat-stem worms.

Lugger (*20, Bull. 43, pp. 210–213*) reported the insect in Minnesota, and recorded evidence which indicated the presence of three generations. Later (*20, Bull. 48, p. 38*) he reported it as damaging late-sown rye, to the extent of 10 percent. Williams (*30, pp. 17–20*) noted its presence in South Dakota. Fletcher (*8*) stated that there were three broods of flies at Ottawa, Canada.

Bruner and Swenk (*1, pp. 24–26*) reported the maggot as being distributed throughout Nebraska. Three broods were recorded, the fall brood usually attacking the grain most severely. Wheat planted in October seemed to be less liable to attack than that sown earlier.

Criddle (*3, pp. 236–237*) collected flies on *Agropyron repens* (L.) Beauv. and *A. occidentalis* Scribn. Cooley (*2*) reported the maggot as injuring wheat in Montana.

Kelly (*17*) reported the laying of eggs by the parasite *Coelinidae meromyzae* within the eggs of *Meromyza americana*. His report states that the parasite eggs hatch within the eggs of *Meromyza* and the young larvae feed within the fatty tissue of the wheat-stem maggots without disturbing their vital processes, emerging as adults from the puparia of *Meromyza*.

Webster (*28*) found the insect in North Dakota but stated that "blighted" heads in wheat may also be due to the foot rot disease. He found the maggot attacking slender wheatgrass (*Agropyron tenerum* Vasey), wild barley (*Hordeum jubatum* L.), and pigeon grass (*Chaetochloa glauca* Scribn.).

One of the best publications on the wheat-stem maggot is that of Gilbertson (*15*). He described the injury produced, recorded the life history of the insect, and described its immature stages. He also gave a list of host plants and furnished information on the control of the pest by a hymenopterous parasite, *Microbracon meromyzae* (Gahan). Gilbertson found an undetermined species of mite attacking the adult flies and an unidentified fungus on dead maggots.

The distribution of *meromyza americana* in Kansas has been given by Sabrosky (*23, p. 216*).

MATERIALS AND METHODS

Detailed studies of the life history of the wheat-stem maggot under Kansas conditions were begun in the fall of 1932. Adult flies were collected and caged on young plants of Turkey wheat (*Triticum aestivum* L., syn. *T. vulgare* Vill.) in the field. These cages were observed daily for evidence of mating and deposition of eggs. After eggs had been deposited the plants were dissected, and the sections bearing eggs were placed in small shell vials and observed daily for evidence of the first hatching. These sections of plants were later examined for young maggots, some of which were preserved for study and others for use in rearing experiments.

The major portion of the life-history studies was carried on in 1933. During the last week of March, maggots secured from clumps of volunteer wheat were placed inside of sections of fresh wheat stems from which the centers had been removed. Each of these sections with the included maggot was placed in a small shell vial in the bottom of which had been packed a thin layer of moist, sifted soil. Each vial was tightly corked, numbered, and recorded. Moisture was added to the soil at frequent intervals to keep the humidity within the vials high, a condition which appeared to be most favorable for the maggots.

In addition to the maggots collected from volunteer wheat, others were collected from winter wheat, spring wheat, barley, rye, and grasses and given similar treatment. Maggots were also reared from eggs secured from the oviposition tests. In all, some 500 maggots were carried through partial or complete life cycles. Of this number, 156 were reared from eggs, and the remainder were collected from various sources and in various larval instars, or as puparia.

Maggots of various stages, eggs, and puparia were preserved for study and measurement. The exuviae from maggots and the cephalopharyngeal skeletons of dead maggots were mounted in Canada balsam on microscopic slides and studied to determine the differences in various instars and to provide the specimens for the drawings of the cephalopharyngeal skeletons.

Early in April sweepings were begun to determine the date of first appearance of the flies. The flies collected in these sweepings were used in the oviposition tests.

In infested fields "blasted"[4] heads appear shortly before harvest (fig. 1, *D*). During the first 2 weeks of June, all of the blasted heads were collected from the wheat variety plots and the date-of-planting-variety plots at the agronomy farm. In making the collections the culms were cut near the crown of the plant so that the entire culm was preserved. The culms from each plot were tied in separate bundles, labeled, and placed in large cardboard boxes, from which the flies were collected as they emerged from the culms. These boxes were closed to the light except for two round holes near the top of one end of each, into which common lamp chimneys were inserted. The outer ends of the chimneys were closed to prevent the escape of the flies as they emerged. A little water was sprayed over the wheat at frequent intervals to insure sufficient moisture for the emerging flies. The boxes were examined daily, and the flies and other insects removed. The sex of each *Meromyza* fly was determined, and any Hymenoptera that might be parasites of *M. americana* were preserved for identification.

After most of the flies had emerged, each of the culms was split lengthwise to determine how many of those having blasted heads had been infested by stem maggots. Of 6,527 culms examined in 1933, 99.9 percent of the blasted heads were due to *Meromyza* injury.

The adult flies from the sweepings and those reared from the culms having blasted heads were used in oviposition tests. Some of the flies were caged in pairs, and others were caged several together on small wheat plants in pots. Common lamp chimneys with cloth tops were used for cages in these experiments in the field insectary. Other

[4] "Blasted" heads are heads which appear white and ripen prematurely with stiff, spreading awns, presenting a striking contrast to the green of the leaves and of the normal heads.



FIGURE 1.—Life stages of and damage produced by the wheat-stem maggot: *A*, Adult female. × 15. *B*, Egg in normal position. × 10. *C*, Maggot in feeding position where central shoot is severed, resulting in blasted heads as shown in *D*. × 3. *D*, Spring type of injury showing blasted heads (*a*) and normal heads (*b*). × ½. *E*, Fall type of injury showing injured tillers, (*a*) and a normal tiller (*b*). × ½.

flies were caged on young wheat growing out of doors. A cage 3 feet square and 1 foot high was used in these outdoor experiments. The primary purpose of the outdoor experiments was to secure large numbers of eggs. In all these experiments records were kept of the number of eggs deposited and the position of the eggs on the plants. These records were compared with those taken on egg-deposition habits in the field.

From time to time records have been made of the infestation of different wheat varieties by the wheat-stem maggot. These counts were made on winter wheats, in the following seasons: Spring of 1921, fall of 1924, 1927, 1931, 1932, and 1933. In each of these variety tests the same number of plants was examined for each variety in the test. This number, however, varied in the different tests, and this variation should be considered in the interpretation of the data.

LIFE HISTORY

ADULT

Meromyza americana may be distinguished from others of its family (Chloropidae) by the fact that the costa extends only to the third vein, and by the greatly enlarged hind femora and the correspondingly curved tibiae (fig. 1, *A*). The flies are slender and pale green to yellow. They are almost devoid of bristles or hairs, with three broad, grayish-black stripes on the mesonotum, three brown to black stripes (which often coalesce) on the dorsum of the abdomen, the antennae slightly infuscated above, and the front almost entirely destitute of hairs except for a few on the orbits and on the lateral margins of the triangle. The second and third veins of the wing are conspicuously curved forward, the latter ending some distance before the apex of the wing. A full technical description is given by Forbes (*9*).

Flies that have recently emerged are pale green in color, but as they grow older the color turns to pale yellow or straw. The eyes vary from a bright green to a deep bronze which variation is probably due to the age of the fly.

The difference in the appearance of the flies of the two sexes is largely in the size, the male being much smaller. The abdomen of the female fly is rounded and swollen, while that of the male is more or less cylindrical, tapering gradually to a dull point.

Soon after the flies emerge from the puparia they begin to mate. Mating may take place at any time during the first few days after emergence, and a single female has been observed mating more than once either with the same or with a different male.

The ratio between the sexes of *Meromyza americana* was determined by examination of 2,149 flies reared from June 10 to June 18. Of these 1,180, or slightly more than half, were females, the ratio being 55 percent females to 45 percent male.

From records kept on 10 females the preoviposition period was found to vary from 2 to 6 days, and the average was 3.6.

EGG

The eggs are snow white, and are fusiform-cylindrical in shape with gently rounded ends. On the anterior extremity is a minute, flattened, rounded area. The surface is covered with longitudinal ridges, the spaces between the ridges being concave and marked off into

rectangular areas by smaller transverse ridges (fig. 1, *B*, and fig. 2, *A*). From measurements made of 50 eggs the length was found to range from 0.96 to 1.09 mm, and the greatest breadth from 0.165 to 0.231 mm. The average length was 1.02 mm and the average breadth 0.194 mm (table 1).

TABLE 1.—*Measurements of the various immature stages of the wheat-stem maggot*

Item	Egg (50 individuals)		(25 individuals) first instar		(25 individuals) second instar		(50 individuals) third instar		(44 individuals) puparium	
	Length	Breadth	Length	Breadth	Length	Breadth	Length	Breadth	Length	Breadth
	mm	*mm*	*mm*	*mm*	*mm*	*mm*	*mm*	*mm*	*mm*	*mm*
Maximum	1.09	0.231	1.86	0.57	3.31	0.66	6.47	1.21	5.86	1.10
Minimum	.96	.165	1.61	.31	3.05	.34	6.21	.96	5.54	.82
Average	1.02	.194	1.75	.45	3.20	.50	6.40	1.10	5.70	.91

On the young plant the eggs of the wheat-stem maggot are usually deposited singly either on the leaves or on the leaf sheaths, but in a few instances eggs were found between the leaf sheath and the stem. In the fall a great many eggs are found glued to the stem just above the ground. In the oviposition experiments carried on in the insectary the majority of the eggs were glued to the upper surface of the leaves a short distance from the stem. In most cases the eggs were parallel to the long axis of the leaves and stem (Fig. 1, *B*).

Since the eggs are laid singly and the oviposition period extends over several days it is difficult to estimate the number of eggs laid by a single individual. In cage experiments an average of 10 to 15 eggs were deposited by each female. The largest number deposited by a single female was 30. The eggs were deposited at an average rate of one to four daily. One female, under cage conditions, deposited 13 eggs in a single day. The length of the oviposition period ranged from 4 to 10 days and the average was 6.91 days.

TABLE 2.—*Length of stages of the wheat-stem maggot*

Item	Oviposition period of adult (32 individuals)	Incubation period (31 individuals)	First instar (42 individuals)	Second instar (26 individuals)	Third instar (16 individuals)	Entire larvae stage	Period in puparium (46 individuals)	Egg to adult	Adult (13 individuals)
	Days	*Days*	*Days*	*Days*	*Days*	*Days*	*Days*	*Days*	*Days*
Maximum	10	11	11	28	[1] 23	[1] 62	20	[1] 82	19
Minimum	4	4	2	2	9	13	5	18	2
Average	6.91	6.80	6.19	11.75	14.56	32.10	11.72	43.82	9.38

[1] Does not include overwintering larvae.

The incubation period varied from 4 to 11 days, with an average length of 6.8 days (table 2).

LARVA

The full-grown larva is an active, slender, glassy, pale-green maggot, tapering anteriorly and to some extent posteriorly (fig. 1, *C*). The hooks, which are typical of muscoid larvae, are found beneath the

head and serve to differentiate it from most other larvae that infest wheat except those of the same group. A more complete description has been given by Forbes (*9*).

It was found in these experiments that the larvae pass through three instars. When first hatched they are very small, slender, and white, with neither a head capsule nor feet. Twenty-five newly hatched larvae were measured, and the average length was found to be 1.75 mm and the average width 0.45 mm (table 1).

The larvae of the second instar are somewhat larger than those of the first instar, and are of a very light whitish-green color. Of 25 such larvae measured the average length was 3.20 mm and the average width was 0.50 mm (table 1).

Fifty larvae of the third or last instar were measured and the average length was found to be 6.40 mm and the average width 1.10 mm (table 1). Forbes' (*9*) description was evidently made from specimens of this instar.

From the suckerlike mouth, located on the under surface of the first segments, usually protrudes a pair of black-toothed hooks, the mandibles (fig. 2, *C*). These are supported by and connected to the cephalopharyngeal skeleton, which is a V-shaped structure extending back into the body as far as the third segment. The cephalopharyngeal skeleton is a brownish or blackish scleritized structure and is usually visible through the integument of the larva. It differs in shape in the different instars. In the third instar it is completely developed and the parts are easily distinguished. The skeleton of this instar is represented in Figure 2, *F*, which shows the mandibular, hypostomal, and pharyngeal sclerites and the small arched dentate sclerite completely developed, with the small accessory sclerites attached to the hypostomal sclerite. The dentes on the mandibular sclerite are fully developed and completely scleritized.

The cephalopharyngeal skeleton of the second-instar larva is represented in figure 2, *E*. This skeleton is similar to that of the third instar, except that it is smaller and the dentes are not fully developed or scleritized.

The cephalopharyngeal skeleton of the first-instar larva is represented in figure 2, *D*. This skeleton differs greatly from that of either of the other instars. Not only is it materially smaller, but it is also less completely developed. The dentate sclerite and the two small accessory sclerites are completely absent and the dentes of the mandibular sclerite are poorly developed. The mandibular sclerite is not heavily scleritized as it is in the skeletons of the other two instars.

The first of the three larval instars was found to require a period of from 2 to 11 days, the average being 6.2 days; the second instar required from 2 to 28 days, the average being 11.4 days; the third required from 9 to 23 days, the average being 14.6 days. The entire larval stage therefore required from 13 to 62 days, or an average of 32.1 days (table 2). These results do not include overwintering larvae.

These calculated results from partial life histories, when compared with the results obtained from eight cases in which the larvae were actually reared from egg to adult, were found to be both higher and lower, but the average time was similar. The length of the three instars in the eight actual tests varied from 16 to 40 days, the average time being 27.4 days.

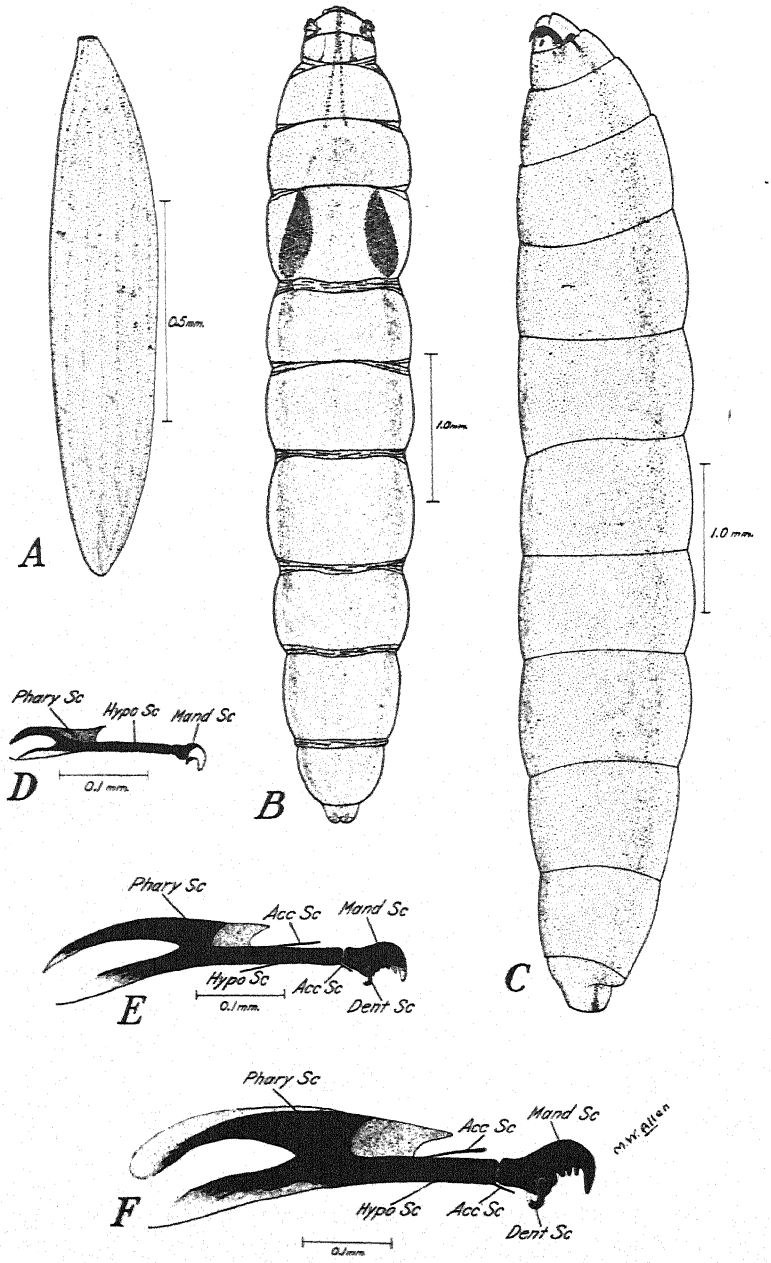


FIGURE 2.—Characteristics of the immature forms of *Meromyza americana*: *A*, Egg; *B*, puparium; *C*, full-grown larva; *D*, cephal opharyngeal skeleton of first-instar larva; *E*, cephalopharyngeal skeleton of second-instar larva; *F*, cephalopharyngeal skeleton of third-instar larva. *phary* Sc, pharyngeal sclerite; *hypo* Sc, hypostomal sclerite; *mand* Sc, mandibular sclerite; *dent* Sc, dentate sclerite; *acc* Sc, accessory sclerite.

PUPA

The pupa forms inside of the last larval skin or puparium, where it may be seen through the semitransparent larval skin. The constrictions between the segments are more marked than in the larva and the puparium is more yellowish in color (fig. 2, *B*). Forbes (*9*) has given a full description of this stage.

Forty-four puparia were measured, and the average length was found to be 5.7 mm and the average width 0.9 mm (table 1). Figure 2, *B*; shows a typical puparium.

The period from the formation of puparia to the emergence of adults varied from 5 to 20 days; the average being 11.7 days (table 2). The time from hatching of the egg to emergence of the adult, calculated from the foregoing results, varied from 18 to 82 days, the average being 43.8 days (table 2). These results were checked against the records of four maggots reared from egg to adult. The four records showed a variation in time from 42 to 57 days with an average of 46.3 days. These figures, both calculated and recorded, are for the spring and summer generations and do not take into consideration the overwintering brood.

SEASONAL CYCLE

From the rearing experiments and from field observations, in the vicinity of Manhattan, the cycle for the year 1933 was determined (fig. 3). The data for 1933 probably represent about the normal for this locality.

The insects overwintered as full-grown larvae which pupated during the latter part of March and the first half of April. The adults began to emerge on April 15 and continued to emerge throughout April and the first half of May. These adults mated within a few days after emergence and deposited their eggs during the latter part of April and through the greater part of May. The eggs began to hatch about the last of April, and the larvae were found in the stems of wheat, rye, and barley throughout May. They pupated about the last of May and in early June. The adult flies emerged from these puparia from June 5 to June 28. The flies of this generation deposited their eggs during the latter part of June, and the larvae emerged from the eggs from June 25 to the last of July. These larvae fed within the stems of wild and cultivated grasses. They pupated from the middle of July until August 8, and the adults emerged until about the middle of August.

Eggs were deposited by this third brood of flies during the latter part of July and the first half of August. The maggots from these eggs emerged during the last few days of July and throughout the greater part of August. They lived in the stems of grasses and volunteer wheat. Many became full-grown larvae, passed the winter in that stage, and pupated the following spring. Some of the larvae from these eggs, however, pupated during August and emerged as adults during the latter part of that month. Adults were present in the field until December 16. These adults, which had emerged during August, September, and the first part of October, deposited eggs that hatched before October 15, the larvae overwintering and pupating the following spring. Thus there is evidence of three full generations and a partial fourth in Kansas.

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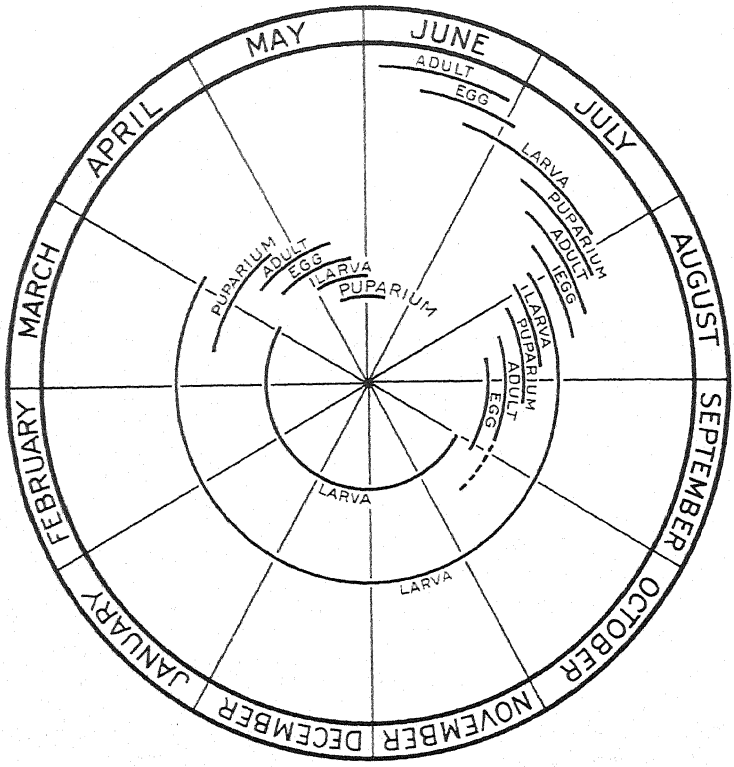


FIGURE 3.—Life cycle of *Meromyza americana*, 1933.

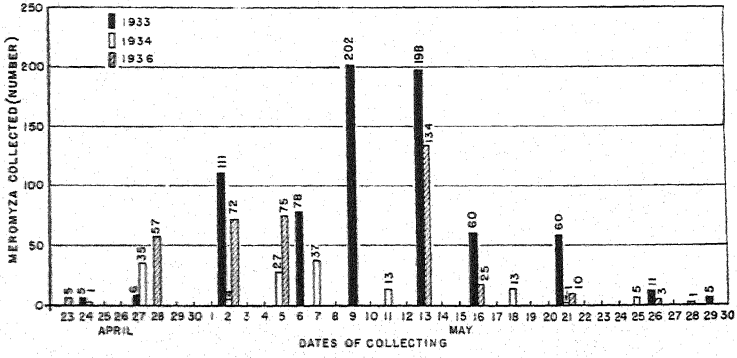


FIGURE 4.—Collection records showing abundance of adults of *Meromyza* during the spring of 1933, 1934, and 1936; these collections were made on grass plots near the wheat plots used in the differential infestation tests.

NATURAL CHECKS

During these experiments 18 species of Hymenoptera were reared, 4 of which were from puparia of *Meromyza americana;* 3 others were from culms of blasted heads, and 11 others were from volunteer wheat known to be heavily infested by the wheat-stem maggot. Table 3 shows nine species which are recorded in literature as parasitic upon *Meromyza*. Only one of these, *Euphoriana uniformis* Gahan, was not reared during these studies. The names of the parasites are listed under their family designations in table 3, with the sources from which reared, the number reared, and literature citations to previous records of their parasitism on *Meromyza*.

Coelinidea ferruginea was described by Gahan (*11*) from three specimens, two females and one male reared by C. N. Ainslie at Elkpoint, S. Dak., the type locality. The host was *Meromyza americana*. All the parasites of this species which were reared during the present experiments were from puparia of overwintering larvae or, in May and June, from the puparia from which the first brood of flies normally emerge.

TABLE 3.—*Hymenopterous parasites of the wheat-stem maggot*

Parasite	Parasites reared from—			Recorded in literature as parasite of *Meromyza*
	Puparia of wheat-stem maggot	Culms having blasted heads infested by maggot	Volunteer wheat infested by maggot	
Ichneumonoidea:				
Alysiidae:	*Number*	*Number*	*Number*	
Coelinidea ferruginea Gahan	10		13	Gahan (*11*).
Coelinidea meromyzae (Forbes)	4		1	Forbes (*9*).
Braconidae:				
Euphoriana uniformis Gahan [1]				Gahan (*11*).
Microbracon mellitor (Say)		1		
Microbracon meromyzae (Gahan)	3	10		Do.
Microplitis melianae Vier		3		
Meteorus vulgaris (Cress.)			1	
Ichnumonidae:				
Casinaria scabriformis Vier			1	
Proctotrupoidea:				
Platygasteridae:				
Leptacis sp			1	
Chalcidoidea:				
Eulophidae:				
Horismenus texanus (Girault)			3	Gahan (*13*).
Notanisomorpha meromyzae (Gahan)			9	Gahan (*13*, p. 226).
Eupelmidae:				
Eupelmus allynii French			2	Gahan (*13*).
Eurytomidae:				
Eurytoma tylodermatis Ashm		1		
Mymaridae:				
Polynema striaticorne Girault			2	
Pteromalidae:				
Bubekia faltax Gahan			15	Do.
Callitula bicolor Spinola			1	Do.
Eupteromalus fulvipes (Forbes)			7	
Halticoptera aenea (Walk.)			1	
Cynipoidea:				
Figitidae:				
Hypodiranchis sp	1		14	

[1] Not reared during these experiments.

Coelinidea meromyzae was described (*9*) from specimens reared from *Meromyza americana* and from specimens collected in the vicinity of Cuba, Ill. Kelly (*17*) gives detailed biological observations on this species. In the present work four specimens were reared in July from puparia produced by maggots of the second generation. The fifth specimen was reared from volunteer wheat in the greenhouse, January 10, probably from a puparium of an overwintering maggot.

Microbracon meromyzae (Gahan) was described by Gahan (*11*) from six specimens, three females and three males, reared by C. N. Ainslie at Elkpoint, S. Dak. A male specimen was also reared by J. A. Hyslop at Hagerstown, Md. The host in both cases was *Meromyza americana*. The three specimens of this species which were reared during the writers' experiments were from puparia formed in late May, early June, and in July. Ten others were reared from the culms of blasted heads.

Hypodiranchis sp. has not previously been reported as attacking the wheat-stem maggot, and, according to L. H. Weld, appears to be an undescribed species. In fact, no species of *Hypodiranchis* seems to have been described from North America; the genotype *H. hawaiiensis*, and most of the known species of the genus were described by William Ashmead from the Hawiian Islands. A related European species *Cothonaspis rapae* (Westd.) has been reported by James (*16*) as parasitic on the cabbage-root maggot (*Hylemyia brassicae* Bouche.). One specimen of *Hypodiranchis* was reared from a single puparium collected June 9, and 14 others were reared from volunteer wheat during October, November, December, and January. Those reared from volunteer wheat probably came from puparia formed by overwintering maggots which matured early under greenhouse conditions.

Other Hymenoptera reared from volunteer wheat or from blasted heads infested by *Meromyza* are listed in table 3. These records show the presence of these species in Kansas and suggest the possibility that some are parasitic on the wheat-stem maggot there, especially where they have previously been reported as parasitic on this insect.

In addition to the hymenopterous parasites of *Meromyza americana* reared during the present experiments, one other has been recorded in the literature. This species, *Euphoriana uniformis*, was described by Gahan (*11*) from the male type reared from *M. americana* by J. A. Hyslop at Hagerstown, Md., and from the female type collected by W. H. Menke at Garden City, Kans.

Frequently reference is made in the literature to a small mite *Pediculoides ventricosus* Newport, which is reported as killing the larvae by sucking their blood. This mite is not known to occur in Kansas.

Many times, both in the rearing experiments and in the field, brown or partially brown larvae were found. They seemed to die slowly, and when the integument was ruptured the internal organs were found to be decayed and liquid. This condition was probably brought about by bacteria.

A fungus determined by C. L. Lefebvre as *Cladosporium* sp. was often found associated with the dead maggots. Most of the species of this genus are saprophytic, although a few are parasitic. This one is probably a saprophyte, but until the species is determined, no definite statement can be made concerning it.

FEEDING HABITS OF THE INSECT AND NATURE OF INJURY TO WHEAT

Upon emerging from an egg the larva makes its way to the point where the central leaf or head-bearing shoot emerges from its investing sheath. Upon reaching this point it works its way down between the sheath and the central shoot, girdling the latter as it progresses downward to its final feeding position, which is at the base of the culm in fall and early spring, and just above the upper node in late spring and summer. The larva enters the central shoot and there does most of its feeding. It feeds by tearing the plant tissues with its mandibles and sucking the juices from the lacerated tissue. Before the larva enter the central shoot the feeding takes place between this shoot and its investing sheath.

When first entering the plant and during the migration down to the feeding position, the larva feeds with its head down, but when the feeding position in the center of the culm is reached this position is reversed, and the greater part of the feeding is done with the anterior end up (fig. 1, *C*).

Two types of injury are produced by the wheat-stem maggot, one in late spring and summer, the other in autumn and early spring. In late spring and summer the larva destroys the lower part of the stem, thus preventing the normal flow of sap to the head of the plant. The head dries prematurely and the kernels, if present at all, are small, shriveled, and unfit for food or planting. When this type of injury occurs the head and central shoot are light straw color rather than green like those of uninfested stems. The awns are stiff, brittle, and spreading, and do not extend upward from the glumes as in normal plants (fig. 1, *D*).

In the fall and early spring the larva attacks the central shoot (fig. 1, *E*), cuts the vascular bundles, and so prevents the flow of the sap into the upper part of the leaf. This injury may be of considerable importance. In some cases the entire tiller turns yellow and dies. After injuring or killing one tiller the larva may migrate to a fresh one. In laboratory experiments the larvae have sometimes been found to migrate from an injured tiller to a fresh one on another plant. These plants have always been in close proximity, however, and no records are available to show the distance that larvae may migrate. Larvae placed on the soil near plants have, with few exceptions, been able to reach the plants and feed within them.

A serious aspect of this autumnal and early spring type of injury is that the larvae which cause the injury to heading wheat later in the spring are produced by flies that emerge from this earlier generation of larvae.

FOOD PLANTS

During the experiments wheat-stem maggots were observed feeding within the stems of the following species of plants:

Common bread wheat (*Triticum aestivum* L.).
Durum wheat (*Triticum durum* Desf.).
Triticum vulgare×*T. durum* (from various sources).
Triticum macha Va.
Triticum persicum stramineum Zhuk.
Triticum timopheevi Ahuk.

Barley (six-row) (*Hordeum vulgare* L.).
Rye (*Secale cereale* L.).
Timothy (*Phleum pratense* L.).
Yellow bristlegrass (*Setaria lutescens* (Weigel) F. T. Hubb.).
Green bristlegrass (*Setaria viridis* (L.) Beauv.).

In addition to these food plants the maggot has been reported in the literature as feeding within the stems of:

Emmer (*Triticum dicoccum* Schrank).
Quackgrass (*Agropyron repens* (L.) Beauv.).
Slender wheatgrass (*Agropyron pauciflorum* (Schwein.) Hitchc.).
Bluestem (*Agropyron smithii* Rydb.).
Bluebunch wheatgrass (*Agropyron spicatum* (Pursh and Smith) Scribn.
Foxtail barley (*Hordeum jubatum* L.).
Canada wild-rye (*Elymus canadensis* L.).
Smooth bromegrass (*Bromus inermis* Leyss.).
Japanese chess (*Bromus japonicus* Thunb.).

Meromyza americana has not been found feeding on oats.

DIFFERENTIAL INFESTATION OF VARIETIES

RELATION OF STAGE OF PLANT MATURITY TO NUMBER OF HEADS BLASTED BY *MEROMYZA*

Some evidence of the presence in wheat varieties of resistance to injury from the wheat-stem maggot has been presented by Dunham (*4*) and by Gilbertson (*15*). The number of blasted heads collected in the various 1/40-acre plots of varieties of winter wheat at the agronomy farm [5] are given in table 4. In all cases the number of infested culms collected from each plot is but a small proportion of the total number of heads present. The relative numbers, however, indicate considerable differences among the varieties represented. These differences may be due (1) to differences in the dates of heading and maturity of the several varieties in relation to the peak number of second-generation adults present and laying eggs, or (2) to varietal differences in palatability or attractiveness to the insect giving rise to actual resistance.

Additional data bearing on this question have been secured from a date-of-planting experiment involving four varieties. These varieties, Early Blackhull, Quivira, Kanred, and Oro, were planted on several dates at intervals of approximately 1 week. The varieties studied in 1933, the dates of planting, and the number of blasted heads collected, together with other information to be discussed later, are given in table 5. The marked differences in each variety in the number of blasted heads formed on plants from the various dates of planting are shown in figure 5, *A*. According to figure 5, *A*, and table 5 Oro planted October 5, Kanred planted October 11, Quivira planted October 18, and Early Blackhull planted October 25, produced the greatest number of blasted heads.

[5] These plots, used in yield tests, are under the supervision of Dr. H. H. Laude, to whom the authors are indebted for permission to collect the material and for data on dates of heading, maturity, and other agronomic characters.

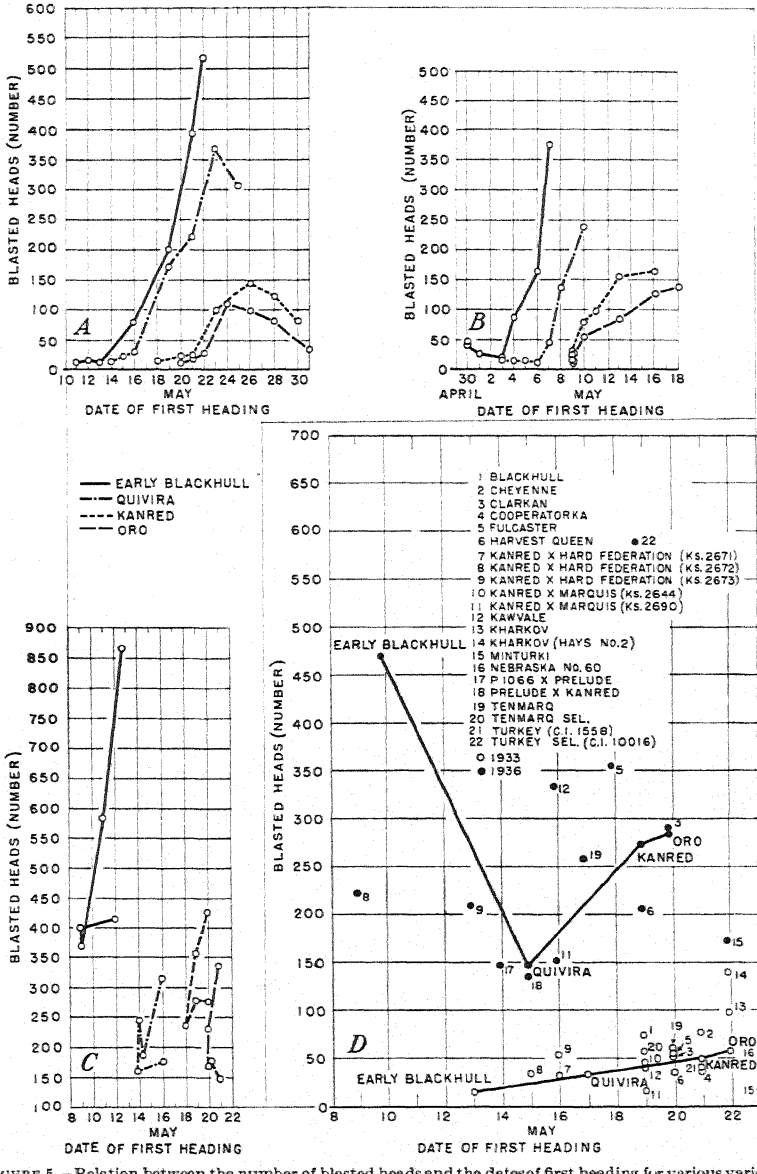


FIGURE 5.—Relation between the number of blasted heads and the datesof first heading for various varieties of wheat in 1933 (*A*), 1934 (*B*), 1936 (*C*), and 1933 and 1936 (*D*).

TABLE 4.—*Relative number of blasted heads produced in wheat varieties by the wheat-stem maggot, spring 1933* [1]

Rank	Kansas no.	C. I. no.[2]	Variety	Date of first heading, May 1933	Blasted heads per 1/40-acre plot				
					Series			Total infested	Average infested
					1	2	3		
					Number	*Number*	*Number*	*Number*	*Number*
1	483	8856	Early Blackhull	13	27	10	9	46	15.3
2	2690	11589	Kanred × Marquis	19	24	9	15	48	16.0
3	2464	6156	Minturki	23	23	21	8	52	17.3
4	2671	11373	Kanred × Hard Federation	16	9	21	64	94	31.3
5	2628	8886	Quivira	17	49	10	36	95	31.7
6	2672	10091	Kanred × Hard Federation	15	40	13	50	103	34.3
7	19	6199	Harvest Queen	20	43	39	22	104	34.7
8	499	8861	Cooperatorka	21	32	63	16	111	37.0
9	570	1558	Turkey	21	39	29	47	115	38.3
10	2593	8180	Kawvale	19	45	38	35	118	39.3
11	2644	10090	Kanred × Marquis	19	44	32	57	133	44.3
12	2401	5146	Kanred	21	50	46	51	147	49.0
13	322	6250	Nebraska no. 60	23	50	69	34	153	51.0
14	505	8858	Clarkan	20	67	57	30	154	51.3
15	317	6471	Fulcaster	20	53	51	55	159	53.0
16	2673	10092	Kanred × Hard Federation	16	73	21	65	159	53.0
17	2670	10089	Tenmarq Selection	19	51	44	69	164	54.7
18	495	8220	Oro	22	43	68	------	111	55.5
19	514	6336	Tenmarq	20	67	73	34	174	58.0
20	343	6251	Blackhull	19	39	89	87	215	71.7
21	2667	8885	Cheyenne	21	32	58	135	225	75.0
22	2591	1442	Kharkof	22	65	139	80	284	94.7
23	2659	6686	Kharkof (Hays no. 2)	22	88	185	------	273	136.5

[1] Heads collected from variety plots of the agronomy farm; varieties planted Sept. 28, 1932.

[2] C. I. denotes accession number of the Division of Cereal Crops and Disease, Bureau of Plant Industry, U. S. Department of Agriculture.

An attempt was made to correlate these differences with other associated facts. Several factors, such as date of emergence of plants, date of first heading, date fully ripe, stand per acre, number of culms per acre, yield, length of fruiting period, plant height, test weight per bushel, rainfall, and temperature were considered. The closest correlation was found between the amount of injury and the date of first heading.[6] Those plots marked "first heading" between May 22 and May 26 had a larger number of blasted heads than those heading either before or after that date. Those plots marked "first heading" after the date of maximum infestation were one plot of Quivira, two plots of Kanred, and three plots of Oro (table 5).

From the data obtained it appears probable that the stage of growth of the plants at the time of oviposition by the flies is important. Apparently in late spring some specific stage in the maturity of the plants is more acceptable to the female flies or is more palatable to the larvae than are other stages of maturity.

In studying another problem Wilbur and Sabrosky (*29*) found the maximum number of adult *Meromyza* on pasture grass less than one-quarter of a mile away from the wheat plots during the period from May 9 to 13. This is 13 days earlier than the date of first heading which marked the maximum infestation in wheat. The period involved is somewhat longer than the average number of days required for incubation of eggs. Apparently the data give an indication of the length of the interval between egg deposition and the time at which the young larvae cause the appearance of blasted heads. This peak of insect emergence, shown in figure 4, appears to be part of the explanation of the differences in infestation associated with different dates of planting.

The figures given do not take into account the number of culms that might have been missed in the count in cases in which the injury took place so early that no part of the blasted head appeared beyond the top leaf sheath. In some of the culms collected the heads were well exserted; in others part of the head was enclosed in the leaf sheath. In each culm collected from these date-of-planting variety plots the distance from the base of the head to the top of the leaf sheath was measured, and the average distance and the percentage of heads completely exserted were calculated (table 7).

These data show differences from plot to plot and between varieties. They also indicate a difference in infestation of nonfruiting culms which should be investigated.

It appears from the data in table 5 that the date of maturity of a variety is one factor but not the only one involved in the differences in infestation by *Meromyza*. For example, there were 379 infested culms in the seven plots of Oro as compared to 1,232 in the plots of Early Blackhull. But the plots of Oro included three which headed after the one with maximum infestation, as well as the three which headed before May 24, while all the plots of Early Blackhull headed before the date which gave the maximum infestation in the case of

[6] First heading is the date when about 10 percent of the plants have headed.

TABLE 5.—*Effect of date of planting and variety of wheat on late spring injury by the wheat-stem maggot in 1933*

Variety	Kansas no.	Blasted heads per 1/10-acre plot and date of first heading of wheat planted														Total blasted heads
		Sept. 14		Sept. 23		Sept. 28		Oct. 5		Oct. 11		Oct. 18		Oct. 25		
		Number		*Number*		*Number*		*Number*		*Number*		*Number*		*Number*		*Number*
Early Blackhull	483	13	May 11	17	May 12	13	May 13	80	May 16	200	May 19	393	May 21	[1] 516	May 22	1,232
Quivira	2628	15	May 14	24	May 15	31	May 16	171	May 19	221	May 21	[1] 366	May 23	305	May 25	1,133
Kanred	2401	16	May 18	23	May 20	24	May 21	98	May 23	[1] 146	May 26	123	May 28	82	May 30	512
Oro	495	10	May 20	20	May 21	28	May 22	[1] 110	May 24	93	...do....	83	...do....	35	May 31	379

[1] Plot in which maximum infestation occurred.

TABLE 6.—*Effect of date of planting and variety of wheat on late spring injury by the wheat-stem maggot in 1934*

Variety	Kansas no.	Blasted heads per 1/10-acre plot and date of first heading for wheat planted														Total
		Sept. 16		Sept. 22		Sept. 29		Oct. 6		Oct. 13		Oct. 20		Oct. 27		
		Number		*Number*		*Number*		*Number*		*Number*		*Number*		*Number*		*Number*
Early Blackhull	483	47	Apr. 30	41	Apr. 30	26	May 1	19	May 3	87	May 5	164	May 7	375	May 8	759
Quivira	2628	17	May 3	16	May 4	15	May 5	10	May 6	45	May 7	137	May 8	237	May 10	477
Kanred	2401	14	May 9	22	May 9	29	May 9	79	May 10	97	May 11	153	May 13	161	May 16	555
Oro	495	12	May 9	10	May 9	24	May 9	53	May 10	84	May 13	125	May 16	135	May 18	443

the other varieties. If the experiment had included plots of Early Blackhull heading at a time comparable to those of Oro, the difference in infestation in favor of Oro might have been much greater.

TABLE 7.—*Average percentage of exserted heads from infested culms collected in date-of-planting variety test and length of peduncle,*[1] *1933*

Date of planting	Oro		Kanred		Quivira		Early Blackhull	
	Percent	Length	Percent	Length	Percent	Length	Percent	Length
		Mm		*Mm*		*Mm*		*Mm*
Sept. 14	40	+16	37	−4	57	+42	46	+14
Sept. 23	38	+3	35	−9	43	+19	65	+42
Sept. 28	50	+9	59	+18	77	+57	85	+54
Oct. 5	52	[2] +14	44	−2	67	+37	76	+40
Oct. 11	8	−41	18	[2] −24	54	+13	76	+47
Oct. 18	18	−26	14	−37	32	[2] −12	56	+13
Oct. 25	11	−36	16	−33	30	−20	66	[2] +33

[1] Measured from base of head to top of the infesting leaf sheath; +=heads extending above leaf sheath, −=heads not surpassing leaf sheath.
[2] Plot in which maximum infestation occurred.

The data from these date-of-planting plots indicate that the relative position of Early Blackhull as the variety with the lowest infestation in table 4 is not in accord with the potentialities of this variety. They indicate further that it is necessary to consider the date of first heading in the respective varieties in interpreting the relative resistance of the varieties. Thus a number of the late-maturing wheats appear to have the highest infestation. But Minturki, which headed within the period indicated as the one in which the highest infestation took place, is probably the most resistant of the wheats listed and perhaps materially better than Oro.

Data collected on these same plots in the abnormally dry season of 1934 (table 6) tend to confirm the results discussed above. The growing season of 1934 was one in which the early plant development was greatly accelerated. All plots of a given variety headed earlier in 1934 than plots of the same variety in 1933. On the other hand, insect development was not so greatly accelerated. The peak of emergence of *Meromyza*, according to the data of Wilbur and Sabrosky (*29*), occurred about May 5 to 7, only about 4 to 6 days earlier than in 1933. This difference between insect and plant development in the 2 years appears to have resulted in a reduction in the difference in total number of blasted heads between the varieties with high and low infestations. There are no plots of any of the varieties which headed later and in which the number of blasted heads was below the maximum per plot for the variety. The rank of the varieties in respect to the plots of maximum infestation in 1934 (table 6) was the same as in 1933 (table 5). The curves (fig. 5, *B*) from the data were also similar to the parts of the curves for the earlier plots of the year 1933 (fig. 5, *A*). The similarity between the results of the 2 years indicates that the differences between the varieties are significant.

TABLE 8.—*Relative number of blasted heads produced in wheat varieties by the wheat-stem maggot, spring 1936* [1]

Rank	Kansas no.	C. I. no.	Variety	Date of first heading, May 1936	Blasted heads per 1/10-acre plot				
					Series			Total infested	Average infested
					1	2	3		
					Number	*Number*	*Number*	*Number*	*Number*
1	2689	11591	Prelude × Kanred	15	58	209	.....	267	133.5
2	2628	8886	Quivira	15	100	168	162	430	143.3
3	2695	11590	P1066 × Prelude	14	94	194	.....	288	144.0
4	2690	11589	Kanred × Marquis	16	.....	148	.....	148	148.0
5	2464	6156	Minturki	22	168	.....	.....	168	168.0
6	19	6199	Harvest Queen	19	201	.....	.....	201	201.0
7	2673	10092	Kanred × Hard Federation	13	.....	230	183	413	206.5
8	2672	10091	Kanred × Hard Federation	9	197	306	159	662	220.6
9	514	6936	Tenmarq	17	254	.....	.....	254	254.0
10	2595	8220	Oro	20	280	.....	.....	280	280.0
11	2401	5146	Kanred	19	196	407	207	810	270.0
12	505	8858	Clarkan	20	284	.....	.....	284	284.0
13	2593	8180	Kawvale	16	336	318	330	984	328.0
14	317	6147	Fulcaster	18	443	252	.....	695	347.5
15	483	8856	Early Blackhull	10	278	587	508	1,373	457.6
16	2720	10016	Turkey	18	.....	579	.....	579	579.0

[1] Heads collected from variety plots of the agronomy farm; varieties planted Oct. 1, 1935, except Minturki, planted Oct. 2, and Oro planted Oct. 4, 1935.

TABLE 9.—*Effect of date of planting and variety of wheat on late spring injury by the wheat-stem maggot in 1936*

Variety	Kansas no.	Blasted heads per 1/10-acre plot and date of first heading for wheat planted—										Total
		Sept. 13		Sept. 20		Sept. 30		Oct. 4		Oct. 11		
		No.		*No.*		*No.*		*No.*		*No.*		*No.*
Early Blackhull	483	464	May 12	452	May 9	419	May 9	633	May 11	916	May 13	2,884
Quivira	2628	226	May 16	210	May 14	294	May 14	237	May 14	365	May 16	1,332
Kanred	2401	325	May 20	327	May 19	286	May 18	405	May 19	476	May 20	1,819
Oro	495	198	May 21	227	May 20	219	May 20	280	May 20	385	May 21	1,309

The lower infestation in 1933 in plots first heading after May 26 may indicate a stage in the growth of culms in which they are more acceptable to the fly for oviposition or more palatable to the young larva. Observations made in earlier years on date-of-planting plots containing the single variety Kanred showed an infestation similar to that on plots of this variety in 1933.

Additional counts of blasted heads in variety and variety-date-of-planting plots were made in 1935 and 1936. The early spring of 1935 was dry, but this was followed by a period of wet weather when the wheats were heading. Some varieties, especially the early ones, sent up a second growth of heads which were heavily infested by *Meromyza*. During the latter part of the time in which blasted heads were being collected it became impossible to distinguish such heads from the earlier matured normal heads. For this reason collection was discontinued before harvest. However, the data secured give further indication of a relation between stage of plant maturity and infestation.

Data secured from counts of blasted heads made in 1936 are recorded in tables 8 and 9 and figure 5, *C*, *D*. Data on abundance of *Meromyza*

are given in figure 4. The dates of first heading of the plots studied are more nearly comparable to those in 1934 than to those in 1933, while the reverse is true of the abundance of *Meromyza*, which appeared in numbers earlier and reached a peak later in 1936 than in 1934. This may have influenced the total number of blasted heads collected.

Some peculiarity of the season of 1936 caused one or more of the earlier planted plots to reach the stage of first heading after some of the later planted ones. In general the number of blasted heads shows a closer relationship to date of first heading than to date of planting, thus giving further evidence of the relation of stage of plant maturity to infestation by *Meromyza*.

The type of season also resulted in bringing the date of first heading for the different dates of planting much closer together than usual, as is indicated in table 10. There is some evidence that the type of season had a different effect on different varieties. Taking these facts into consideration, the data from the variety-date-of-planting plots in 1936 are reasonably similar to those secured in 1933 and 1934.

TABLE 10.—*Range in number of days from earliest date of first heading to latest date of first heading for each of four varieties tested in date-of-planting-variety plots during 1933, 1934, and 1936*

Variety	Range in days		
	1933	1934	1936
Early Blackhull	11	8	4
Quivira	11	7	2
Kanred	12	7	2
Oro	11	9	1
Total range of all varieties	20	18	12

In the variety series (table 8) the relative infestation of early maturing varieties in general is somewhat different from that of 1933 under a lighter infestation. Especially is this true of the relationship of Early Blackhull and Quivira to the other varieties. On the other hand, Minturki still retains its position with the lowest infestation when the period of first heading is taken into consideration. Four varieties heading at an earlier date than Minturki had a lower infestation but were not exposed to the maximum oviposition. Hence, throughout these spring infestations there is evidence that both variety and stage of maturity are important in determining the amount of injury.

The results of both variety and date-of-planting tests indicate the value of an early maturing variety or early maturity in the lessening of damage by the spring brood of the wheat-stem maggot.

FALL INFESTATIONS

From a summary of counts of maggot infestation made during several years previous to 1934 it was found that some varieties of wheat appeared to have less of the fall and early-spring type of injury than did other varieties. Among 80 varieties and strains of wheat studied in two or more tests there was a range of from 0 to 29 in the average percentage of plants infested. Most of the varieties having a low infestation had been in a very few tests in which the intensity

of the infestation was low on all varieties. Hence these low figures are considered to be of doubtful significance. The intensity of infestation in the different years, as indicated by the average infestation, varied from 2.8 to 32.6.

From among this group of wheats the 21 varieties recorded in table 11 have been selected for presentation because they were studied in five or more tests or were of particular interest in connection with the study of the late spring infestation.

TABLE 11.—*Infestation of winter-wheat varieties by the fall generation of "Meromyza americana" in various years between 1924 and 1933*

Kansas no.	C.I. no.	Variety	Early spring 1921, 25 plants	Fall 1924, 15 plants	Fall 1927, 20 plants	Fall 1931, 60 plants	Fall 1932, 50 plants	Early spring 1933, 25 plants	Fall 1933, 50 plants	Average infestation of—	
										Variety named	Turkey in same tests
			Per cent	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*
2522	6161	Honor	.....	0.0	0.0	10.0	8.0	0.0	.....	3.6	13.8
2564	3342	Dawson	.....	.0	10.0	3.5	10.0	.0	.....	4.7	13.8
19	6199	Harvest Queen	24.0	6.8	.0	4.0	.....	.0	.....	6.9	20.6
439	6936	Tenmarq	.....	6.8	5.0	4.0	4.0	.0	28.0	9.1	17.8
2448	6155	Minturki	.....	.0	15.0	.....	12.0	.....	14.0	10.3	24.8
2672	10091	Kanred×Hard Federation	.....	.....	.....	13.0	6.0	5.9	28.0	10.7	13.0
2401	5146	Kanred	.....	7.2	8.9	2.5	2.0	4.0	44.0	11.4	17.8
435	6163	Shepherd	.....	6.8	10.0	25.0	18.0	.0	.....	12.0	13.8
223415	.....	Illini Chief Selection	.....	9.2	8.8	1.3	6.0	8.0	44.0	12.9	17.8
2667	8885	Cheyenne	.....	.....	.....	14.1	8.0	.0	30.0	13.0	13.0
483	8856	Early Blackhull	.....	.....	.....	25.8	10.0	.0	20.0	13.9	13.0
2132	.....	Red Winter	28.0	.0	30.0	14.0	12.0	4.0	.....	14.7	18.2
2628	8886	Quivira	.....	.....	.....	25.4	8.0	.0	26.7	15.0	13.0
2993	8180	Kawvale	.....	.....	15.0	1.9	22.0	8.0	28.0	15.0	16.8
2588	5338	Imperial Amber	.....	33.3	10.0	14.0	6.0	12.5	.....	15.2	13.8
495	8220	Oro	.....	.....	.....	.....	12.0	.0	34.0	15.3	16.0
2594	8257	Fulhard	.....	.....	30.0	9.3	10.0	8.0	28.0	17.1	16.8
	5566	Beechwood	32.0	13.3	25.0	20.0	14.0	.0	.....	17.4	18.2
343	6251	Blackhull	.....	.....	12.3	13.8	12.0	10.5	44.0	18.5	16.8
359	5597	Red Rock	.....	.0	25.0	40.0	12.0	4.5	32.0	18.9	17.8
570	1558	Turkey	40.0	40.0	15.0	4.0	6.0	4.0	38.0	21.0	21.0
		Average	32.6	7.1	13.1	11.5	7.8	2.8	32.3	.....	.....

A few varieties, such as Turkey, Red Rock, Blackhull, and Beechwood, were rather consistently high in all tests. Other varieties, such as Honor, Dawson, Harvest Queen, Tenmarq, and Minturki, were usually below the average in all tests.

It is of interest that the variety Minturki, which showed little infestation by the late-spring brood, also showed relatively little infestation by the fall brood. The varieties Oro, Kanred, Quivira, and Early Blackhull, studied in the date-of-planting series are not arranged in the same ranking under the two infestations. From the small differences between varieties and the erratic behavior in the different years it is questionable whether the low fall varietal infestation here indicated is of economic importance. However, additional tests under more uniform infestation may show differences of importance.

SUMMARY

In the life history of *Meromyza americana* Fitch there are three larval instars which are distinguishable by the shape of the cephalopharyngeal skeleton. The total life cycle is completed in from 18

to 82 days in the summer. The winter is passed in the larval stage. At Manhattan, Kans., there are three generations and a partial fourth each year.

During the course of these experiments four parasite, *Coelinidea ferruginea* Gahan, *C. meromyzae* (Forbes), *Microbracon meromyzae* (Gahan), and *Hypodiranchis* sp., were reared from purparia of the wheat-stem maggot. Eighteen species of Hymenoptera, including these four, were reared from volunteer wheat or culms heavily infested by the wheat-stem maggot. Nine species known to be parasitic on *Meromyza* are recorded in the literature.

In the fall and early spring the wheat-stem maggot kills the central leaf of the plant destroying the tiller on which it feeds. The white or blasted heads which appear at heading time result from the feeding of the larva above the upper node.

The wheat-stem maggot has been recorded as feeding on several species of *Triticum* on barley, rye, and on a number of native and introduced grasses.

Plants from later sown seeds were more heavily infested in the spring than plants from earlier sowings. Differences in resistance to infestation were noted among the different wheat varieties tested. These differences may not be of economic importance but further study should be made under more frequent and heavier infestations.

The data collected from the variety and date-of-planting plots indicate that there is a stage in the growth of the plant which is more palatable to the larva or more attractive to the female fly than are other stages of growth.

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COMPARATIVE CONFORMATION, ANATOMY, AND UDDER CHARACTERISTICS OF COWS OF CERTAIN BEEF AND DAIRY BREEDS[1]

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The amount of secretory tissue in the udder has been shown by experiment to be very much less in a highly specialized beef cow than in a highly specialized dairy cow, even though the internal anatomy and the skeletal structure of the two cows are surprisingly similar (*8*).[3] These results suggested the possibility that the difference in amount of secretory tissue in the udder, together with differences in persistency of lactation, may constitute the chief hereditary characteristics responsible for the wide difference in milk-producing capacity which has long been known to exist between cows of the dairy and beef breeds.

Data on the quantity of secretory tissue in the udder were available for a large number of dairy cows whose milk-producing capacity and persistency of lactation had been determined. Similar data on the amount of secretory tissue in the udder of a beef cow, however, were limited to the individual used in the comparison mentioned (*8*). It seemed desirable, therefore, to determine whether or not the udder of the beef cow studied was typical of the udders of cows of the specialized beef breeds, and also to measure the producing capacity and the persistency or length of lactation of such beef cows when kept through their first lactation period under the same conditions of feeding, milking, and general management as dairy cows.

In order to make such a study possible a cooperative arrangement was made with the Animal Husbandry Division, Bureau of Animal Industry, in 1927, whereby that Division was to provide at the Iberia Livestock Experiment Farm, Jeanerette, La., a group of eight bred heifers of one of the beef breeds, suitable for such a study. In order to avoid as far as possible the influence of any inheritance of dairy-breed characters on milk-producing capacity, persistency of lactation, and mammary development, it appeared desirable to use beef animals that were registered or from registered parents, and that were of similar breeding. Animals of uniform size and condition, about 2 years of age and bred to calve within about 3 or 4 months, seemed most suitable for this study.

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[3] Reference is made by number (italic) to Literature Cited, p. 286.

The Bureau of Dairy Industry, for its part, agreed (1) to keep four of the animals through one lactation period under conditions of feeding, milking, and management comparable to those under which dairy animals of similar age and stage of lactation are kept at that station; (2) to keep the records of their production; (3) to obtain ante-mortem photographs and body measurements; (4) to obtain post-mortem anatomical data at the time of slaughter; and (5) to make a comparative study of the ante-mortem characteristics, the size, the capacity, and the gross anatomy and histology of their udders, according to the method regularly employed in studying the udders of dairy animals. These four animals were to be placed in the dairy barn at least 2 months before freshening, confined in stanchions, and fed and handled regularly to accustom them to the conditions of dairy-herd management before freshening, in order to avoid any rapid and permanent decline in milk flow which might result from a radical change in management at the very beginning of lactation. During the preliminary period in the dairy barn they were to be fed liberally to produce good development and conditioning without excessive fattening. Each animal was to be weighed every 28 days throughout the entire period of the experiment. After freshening, each cow in this group was to be milked twice daily; the quantity of milk produced at each milking was to be recorded, and a 2-day butterfat test was to be made each month. The ration was to be adjusted every 14 days and every effort was to be made to maintain lactation on a maximum plane and to continue it as long as possible. Breeding was to take place not earlier than 3 months and not later than 5 months after calving in order that the effect of gestation might not be a disturbing factor in comparing the quantity of milk produced or the persistency of lactation of cows of the beef and dairy breeds when kept under comparable conditions of dairy-herd management.

The other four animals, of similar breeding, size, age, condition, and gestation, were to be placed in the beef herd on the same experimental farm, and kept under conditions of management prevailing in that herd to show whether or not the difference in feeding and the regularity of milking beef cows kept under dairy-herd conditions would appreciably affect the size, capacity, abundance of secretory tissue, and gross and microscopic anatomy of their udders. On freshening, these animals would be permitted to run with their calves and would be fed the same as other cows in the beef herd. No attempt was to be made to measure milk- and butterfat-producing capacity or persistency of lactation, or to prolong the lactation period. Breeding was to take place according to the schedule usually followed in the beef herd. The only records to be kept on this group were those concerning breeding, calving, and live weight. After each animal had completed her lactation period, the conformation, anatomy, and comparative udder data were to be obtained according to the plan outlined for the group kept under dairy-herd conditions.

ANIMALS USED AND PROCEDURE FOLLOWED

In October 1927 three Hereford heifers (H–15, H–16, and H–17) from registered parents, all by the same sire and born in January 1926, were purchased from a Louisiana breeder. A search for additional animals suitable to make up the two groups was not immediately successful. Meanwhile the three animals were delivered at

the experimental farm and placed in the dairy barn. H–15 died from the effects of conditions induced by abnormal presentation of the fetus, and does not furnish any of the data on which this study is based.

The 2-day average weights of H–16 and H–17, as taken on October 27 and 28, 1927, were 720 and 695 pounds, respectively. On October 27 their ages were 21 months 22 days and 20 months 29 days. So far as the investigators know, normal weights for growing Hereford heifers have not been published. The average weight of these two animals, based on the 2-day average taken at the ages indicated, was 708 pounds. The herd in which H–16 and H–17 were raised received more individual attention than most herds in the vicinity, and the heifers purchased for the experiment were unusually tame and easily handled. They soon became accustomed to their new surroundings and conditions of management. They increased in weight and their general condition and behavior were satisfactory.

The three animals in this first lot were supposed to have been pasture-bred and pregnant when purchased. Breeding dates were not available except that none of the animals had been observed in heat since July 1927. However, on December 14, 1927, H–17 came in heat and was bred. H–16 was pregnant when purchased and calved normally on June 26, 1928. The loss of H–15 through death and the rebreeding of H–17 left only one of the animals originally purchased in suitable condition for use in the milking phase of the experiment. Consequently six additional Hereford heifers, 2 years of age and from registered parents, were selected in southern Texas. They were pasture-bred.

These heifers had been "roughing it" on pasture during the winter. They were shipped to Jeanerette, La., and during the first few days after their arrival they were kept tied, their horns were tipped, and they were branded. When examined, photographed, weighed, and measured on April 7, 1928, about a week after their arrival, they were thin and gaunt. They were below the average in size and condition for a breeder's herd, but were classed as good range cattle for the Gulf Coast region. Identification numbers were assigned to these heifers as follows: H–1, H–2, H–3, H–4, H–5, and H–6. Their weights on April 7 were, respectively, 550, 600, 580, 580, 625, and 595 pounds. Eighteen days later, on April 25, their weights were, respectively, 615, 660, 640, 645, 650, and 695 pounds. The minimum gain during that brief period was 25 pounds, the maximum 100 pounds, and the average 62.5 pounds. The weights of H–16 and H–17, on April 9, were 980 and 930 pounds. They had gained 260 and 235 pounds, respectively, since their arrival at the experiment station farm.

On June 4, 1928, the six heifers purchased in Texas were divided into two groups of three each on the basis of the uniformity of stage of gestation as determined by rectal examination. This basis for selection was used in order that those to be milked in the dairy barn might all freshen at about the same time and be in milk during the same period, thereby insuring more comparable conditions and facilitating the work required in carrying on the experiment. The pregnancy examination indicated that H–2, H–5, and H–6 were most nearly uniform and most advanced in stage of gestation, and they were selected for the group to be kept under dairy-herd conditions. Because of lack of uniformity in gestation, H–1, H–3, and H–4 were placed in the group to be kept under beef-herd conditions. In addition to the six heifers

purchased in Texas, two of the three animals originally purchased in Louisiana were still available, and in properly balancing the two groups it seemed desirable to place one of these older heifers in each group. H–16 was pregnant and apparently due to freshen within a month. H–17, though pregnant from a breeding on December 14, 1927, had reacted later to the agglutination test for infectious abortion. Since H–16 was more advanced in gestation and negative to the abortion test, she was placed in the group to be milked in the dairy barn, and H–17 was placed in the group to be kept under beef-herd conditions.

The group of Herefords consisting of H–2, H–5, H–6, and H–16, selected to be placed in the dairy herd, milked, and kept under conditions of dairy-herd management will subsequently be referred to as group 1. The group consisting of H–1, H–3, H–4, and H–17, selected to be kept in the beef herd under conditions of beef-herd management will be referred to as group 2.

Similar data for the Aberdeen Angus cow A–300 (*8*) and for two registered Holstein-Friesian cows (no. 299 and no. 827) were used for comparison with those obtained on the Herefords.

Figures 1, 2, and 3 show the animals used in this study.

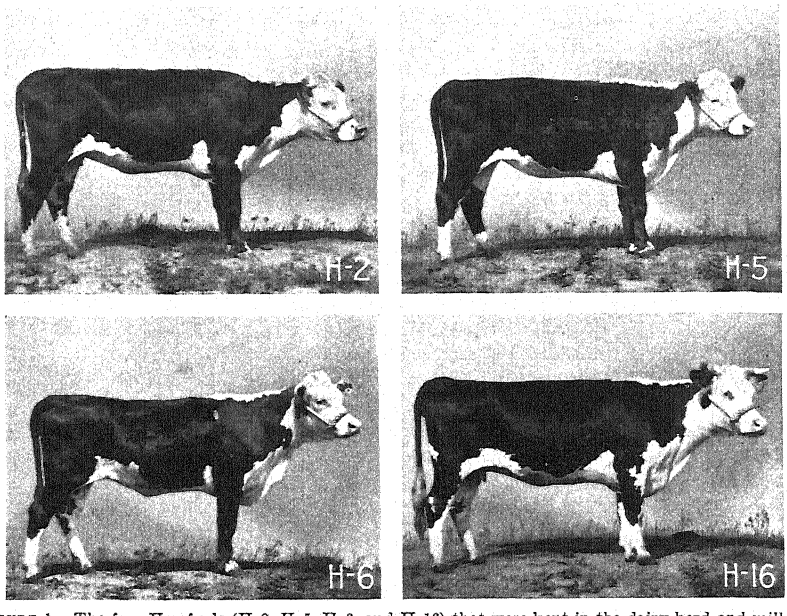


FIGURE 1.—The four Herefords (H–2, H–5, H–6. and H–16) that were kept in the dairy herd and milked through one lactation period.

THE HEREFORD EXPERIMENT

The details of the Hereford experiment were carried on essentially as outlined. The heifers in group 1 (dairy management) adjusted themselves slowly to the conditions prevailing in the dairy herd. Though not so gentle as animals raised under dairy conditions, they caused comparatively little trouble with the exception of occasional kicking. All of them calved normally. The heifers in group 2 (beef management) did not follow the schedule so well. The calving and breeding records of each of the animals in both groups are given in table 1.

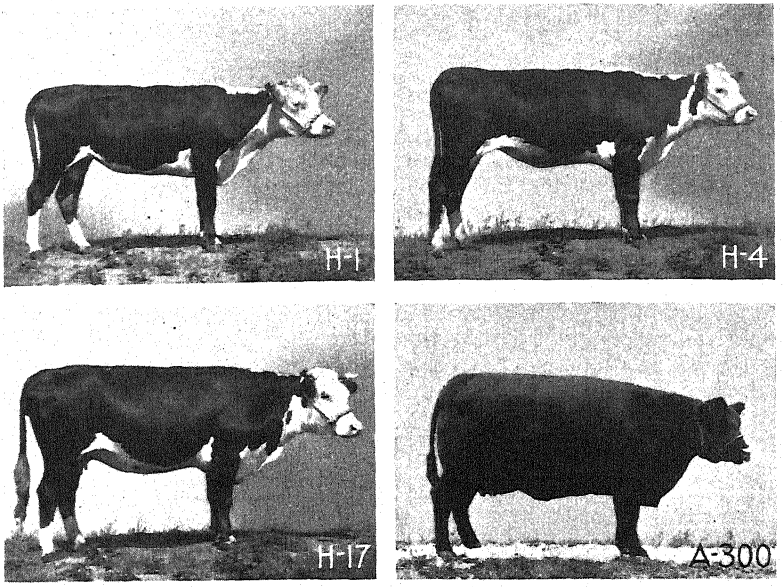


FIGURE 2.—The three Herefords (H-1, H-4, and H-17) that were kept in the beef herd; and the Aberdeen Angus cow (A-300) used for comparison.

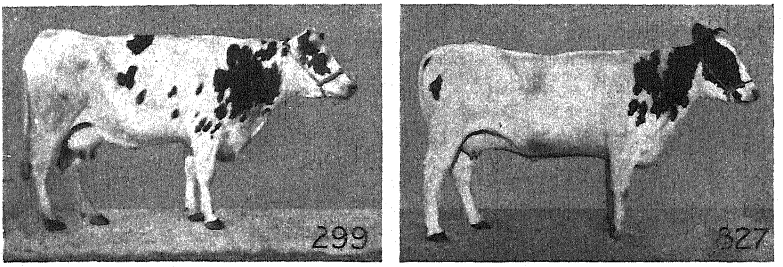


FIGURE 3.—The two Holstein-Friesian cows (no. 299 and no. 827) with which the Herefords and the Aberdeen Angus cow were compared.

TABLE 1.—*Calving and breeding records of Hereford heifers*

Group, and animal no.	Date of calving	Calving notes	Date rebred	Bred to—
Group 1 (dairy management):				
H-2	Oct. 1, 1928	Bull calf	Jan. 12, 1929	Hereford.
H-5	Oct. 17, 1928	Heifer calf	Apr. 9, 1929	Jersey.
H-6	Sept. 22, 1928	do	Mar. 21, 1929	Do.
H-16	June 26, 1928	do	Nov. 9, 1928	Hereford.
Group 2 (beef management):				
H-1	Dec. 14, 1928	Bull calf	(1)	
H-3 [2]				
H-4	Dec. 20, 1928	Aborted	(3)	Shorthorn.
H-17	June 12, 1928	do	Sept. 19, 1928	Brahman.

[1] Not bred. Nursed calf until late in May 1929.
[2] Died July 19, 1928. Disease diagnosed as anaplasmosis.
[3] Pasture-bred, after Mar. 1, 1929.

BODY WEIGHTS AND FEED CONSUMPTION

Every animal in group 1 was substantially heavier at the time of her final weight at the station on May 29, 30, and 31, 1929, than at the beginning of the experiment. Similar gains were made by the

animals in group 2 with the exception of H–1, which nursed her calf until a short time before the termination of the experiment. Each of the four animals in group 1 increased in weight during lactation, but remained nearly constant in weight after the completion of lactation. Two of the animals in group 2 made gains of approximately 100 pounds after calving, but H–1 showed a slight loss. The calf born to H–1 weighed 35 pounds at birth as compared with an average birth weight of 51 pounds for the calves born to the cows in group 1. All the calves were kept until they were at least 4 months old, when H–1's calf weighed 100 pounds as compared with an average of 160 pounds for the other four. So far as is known H–1's calf received nothing but its mother's milk and what feed it might have obtained while running with the herd. The other four calves were given whole milk for 30 days, and then skim milk. Grain and hay were first given at 1 week of age, the quantity being increased until the calves were consuming 3 pounds of grain and 3 to 4 pounds of hay daily at 3 months of age. The individual live weights for both groups of cows are given in table 2.

TABLE 2.—*Live weights of Herefords* [1]

Date	Group 1 (dairy management) [2]				Date	Group 2 (beef management) [3]		
	H–2	H–5	H–6	H–16		H–1	H–4	H–17
	Pounds	*Pounds*	*Pounds*	*Pounds*		*Pounds*	*Pounds*	*Pounds*
Oct. 27 and 28, 1927	--------	--------	--------	720	Oct. 27 and 28, 1927	--------	--------	695
Nov. 9	--------	--------	--------	770	Nov. 9	--------	--------	750
Dec. 7	--------	--------	--------	790	Dec. 7	--------	--------	780
Jan. 4, 1928	--------	--------	--------	820	Jan. 4, 1928	--------	--------	810
Feb. 1	--------	--------	--------	890	Feb. 1	--------	--------	860
Feb. 29	--------	--------	--------	910	Feb. 29	--------	--------	889
Mar. 28	--------	--------	--------	880	Mar. 28	--------	--------	890
Apr. 7 or 9	600	625	595	980	Apr. 7 or 9	550	580	930
Apr. 25	660	650	695	930	Apr. 25	615	645	950
May 23	625	590	650	960	May 23	565	602	980
June 20	715	685	730	970	June 6	620	630	990
July 18	755	715	773	915	July 4	625	700	1, 000
Aug. 16	745	710	772	902	Aug. 1	660	735	990
Sept. 12	780	735	805	922	Aug. 29	690	730	980
Oct. 10	695	720	680	913	Sept. 26	710	740	1, 010
Nov. 7	745	665	725	942	Oct. 24	705	745	1, 000
Dec. 5	720	700	760	950	Nov. 21	760	780	1, 020
Jan. 2, 1929	760	710	790	970	Dec. 19	650	710	995
Jan. 30	750	705	760	955	Jan. 16, 1929	660	710	995
Feb. 27	790	735	790	995	Feb. 13	640	670	1, 000
Mar. 27	760	690	730	940	Mar. 13	570	690	970
Apr. 24	760	730	780	960	Apr. 10	610	785	1, 050
May 22	840	760	810	950	May 8	650	820	1, 050
May 29, 30, and 31 [4]	835	775	792	953	May 29, 30, and 31 [4]	615	833	1, 100
June 13 [5]	815	760	810	965	June 13 [5]	595	840	1, 115
June 15 [5]	780	770	780	955	June 15 [5]	590	825	1, 080

[1] H–15 and H–3 died during the early part of the experiment, consequently their weights are omitted.
[2] The heavy cross rules indicate the beginning and the end of the lactation period.
[3] The heavy cross rules indicate the beginning of the lactation period. The termination of the period is not definitely known.
[4] At Jeanerette, La.
[5] At Benning, D. C.

The four animals in group 1 were fed according to their calculated requirements (Savage standard plus 10 percent).

Table 3 shows the amounts of the various feeds consumed during the experiment. The hay was lespedeza, the silage was sorghum and soybean a part of the time and corn and soybean the remainder of the time. The grain mixture consisted of the following feeds: Brewers' rice, cottonseed meal, wheat bran, rice bran, rice polish, corn-and-cob meal, bone meal, and salt.

The cows were on pasture from June 21 to November 30, 1928, and from March 14 to May 29, 1929. From March 14 to 27, 1929, they were on pasture only half of each day.

The ration was the same as that fed the station's dairy herd during the same period.

TABLE 3.—*Feed consumed by Herefords in group 1 (dairy management) by 28-day periods (1928–29)*

Feeding period	H–2				H–5				H–6				H–16			
	Grain	Hay	Silage	Pasture	Grain	Hay	Silage	Pasture	Grain	Hay	Silage	Pasture	Grain	Hay	Silage	Pasture
	Lb.	*Lb.*	*Lb.*	*Dys.*	*Lb.*	*Lb.*	*Lb.*	*Dys.*	*Lb.*	*Lb.*	*Lb.*	*Dys.*	*Lb.*	*Lb.*	*Lb.*	*Dys.*
June 21 to July 18	----	----	----	28	----	----	----	28	----	----	----	28	84	----	----	28
July 19 to Aug. 15	----	----	----	28	----	----	----	28	----	----	----	28	91	----	----	28
Aug. 16 to Sept. 12	----	----	----	28	----	----	----	28	----	----	----	28	84	----	----	28
Sept. 13 to Oct. 10	42	----	----	28	----	----	----	28	35	----	----	28	70	----	----	28
Oct. 11 to Nov. 7	28	----	----	28	28	----	----	28	28	----	----	28	56	----	----	28
Nov. 8 to Dec. 5	28	84	420	23	28	84	420	23	28	84	420	23	56	84	420	23
Dec. 6 to Jan. 2	28	112	805	----	28	112	805	----	28	112	805	----	56	112	805	----
Jan. 3 to Jan. 30	28	140	840	----	28	140	840	----	28	140	840	----	49	140	840	----
Jan. 31 to Feb. 27	28	168	840	----	28	168	840	----	28	168	840	----	42	168	840	----
Feb. 28 to Mar. 27	28	140	330	7	28	140	330	7	28	140	330	7	42	140	330	7
Mar. 28 to Apr. 24	----	----	----	28	----	----	----	28	----	----	----	28	----	----	----	28
Apr. 25 to May 22	----	----	----	28	----	----	----	28	----	----	----	28	----	----	----	28
May 23 to June 19	----	----	----	7	----	----	----	7	----	----	----	7	----	----	----	7

RECORDS OF MILK PRODUCTION

The milk-producing capacity of the animals in group 1 was surprisingly low. Except for one animal, the duration of the lactation period was very brief, although the animals were milked until the quantity of milk obtained at a milking was extremely small, in an effort to continue lactation as long as possible. Furthermore, since no animal was bred until 102 days after calving, and one did not conceive until 180 days after calving, pregnancy could not have been responsible for the rapid decline in milk flow and the brevity of the lactation periods. Feeding a ration calculated to furnish an excess of nutrients was continued until the cows were turned on pasture March 14, 1929, at which time two of the animals (H–2 and H–6) were dry and the other two (H–5 and H–16) were producing respectively 0.8 pound and 3.5 pounds of milk daily. The records of milk and butterfat production are given in table 4.

The data presented in table 4 require but little discussion. The lack of persistency in the case of H–2, H–5, and H–6 is shown by the brevity of the periods of lactation, which were only 153 days, 202 days, and 159 days respectively. Moreover, the monthly production records, the averages for daily production, the averages for the first 10 days of lactation, the maxima for one milking and the maxima for 1 day, all bespeak the extremely low level of production for these three cows. Undoubtedly H–6 was the poorest milk and butterfat producer in the entire group, her total being only 80.6 pounds of milk and 3.73 pounds of butterfat for the entire lactation of 159 days—an average of only 0.51 pound of milk daily. Presumably H–16 was the oldest cow in the group. At any rate she was larger and better developed than any of the others. Though her milk flow was very low from a dairy standpoint it was more abundant and more persistent than that of any other cow in the group, her lactation period continuing for 329

days and her total production amounting to 1,767.8 pounds of milk and 90.52 pounds of butterfat. This is more than 11 times as much milk and 13 times as much butterfat as the average for the other three cows in the group. The fact that H–16 was so superior to the other animals in group 1 may be accounted for partly by her greater size and presumably more advanced age. Since she came from a different line of breeding, her inheritance may have been partly responsible for her higher production.

To confirm the statement previously made to the effect that the lactation period of these animals was prolonged to the extreme, attention is called to the fact that the average daily production for the last 10 days in milk was 0.20 pound for H–2, 0.50 pound for H–5, 0.17 pound for H–6, and 0.94 pound for H–16. On the last 2 days of lactation H–2 and H–6 were milked only once daily.

TABLE 4.—*Milk and butterfat production of Herefords in group 1 (dairy management)*

Year and month	H-2 [1]			H-5 [2]			H-6 [3]			H-16 [4]		
	Milk	Butterfat	Days milked	Milk	Butterfat	Days milked	Milk	Butterfat	Days milked	Milk	Butterfat	Days milked
1928:	*Lb.*	*Lb.*	*No.*	*Lb.*	*Lb.*	*No.*	*Lb.*	*Lb.*	*No.*	*Lb.*	*Lb.*	*No.*
June										8.8	.40	2
July										272.2	12.39	31
August										255.7	10.48	31
September							6.1	.31	3	226.8	10.21	30
October	52.1	3.05	28	34.7	1.41	10	30.1	1.51	31	184.5	10.79	31
November	31.0	1.57	30	57.3	2.32	30	18.3	.93	30	158.4	8.08	30
December	19.1	.86	31	43.9	1.49	31	10.2	.33	31	160.6	7.36	31
1929:												
January	14.2	.74	31	38.6	1.68	31	8.3	.39	31	139.5	7.74	31
February	8.6	.27	28	31.2	1.03	28	6.8	.23	28	114.8	7.00	28
March	1.0	.03	5	23.6	.78	31	.8	.03	5	117.1	7.08	31
April				20.5	.90	30				96.1	6.73	30
May				5.6	.25	11				33.3	2.26	23
Total	[5] 126.0	6.52	153	[6] 255.4	9.86	202	[7] 80.6	3.73	159	[8] 1,767.8	90.52	329
Daily average production:												
Entire period	.82			1.26			.51			5.37		
First 10 days	1.95			3.47			1.34			7.32		
Last 10 days	.20			.50			.17			.94		
Maximum production:												
For 1 milking	1.5			2.3			1.4			5.2		
For 1 day	2.6			4.1			2.4			10.1		

[1] Calved Oct. 1, 1928.
[2] Calved Oct. 17, 1928.
[3] Calved Sept. 22, 1928.
[4] Calved June 26, 1928.
[5] Butterfat test, 5.17 percent.
[6] Butterfat test, 3.86 percent.
[7] Butterfat test, 4.63 percent.
[8] Butterfat test, 5.12 percent.

During the time the Herefords were on experiment (June 26, 1928, to May 23, 1929) eight first-lactation Jerseys in the same herd were being tested for milk and butterfat production. Three of the Jerseys were in milk following abortions, whereas all of the Herefords in the group calved normally. Moreover, two and probably three of the Jerseys had had cattle-tick fever prior to going on test, contracted as a result of moving the herd from the experimental farm because of the flood. On the other hand, the Jerseys were slightly older when they freshened (average 3 years 1 month) and were milked three times daily during at least a part of the lactation period. The average production of the eight Jerseys was 6,684 pounds of milk and 358

pounds of butterfat. This is approximately 12 times the average milk production and approximately 13 times the average butterfat production of the four Herefords.

It is recognized that the Hereford is a specialized beef breed that has been bred for meat rather than dairy purposes. The Hereford cow consequently is expected to be a low milk producer, yet she seldom fails to raise a calf successfully without the aid of nurse cows—even under range conditions.

When compared with cows of a dairy breed of similar age, the production records of the four Herefords were extremely low. The animals in this group apparently did not possess the capacity to produce liberal quantities of milk, or the stimulus to continue lactation over an extended period. The fact that three of these animals were undersized is recognized, but with the opportunities afforded them both before and during lactation, they could hardly have failed to make a more creditable showing if their inheritance had included the capacity for abundant lactation.

BREED COMPARISONS

Since this study of the conformation, anatomy, udder characteristics, and lactating capacity of Herefords was prompted by results obtained in comparing the udder development of the Aberdeen Angus cow A–300 with that of the Jersey cow Sophie 19th of Hood Farm (*8*), it seemed fitting that an attempt be made to determine whether the conditions found in the udder of the Aberdeen Angus cow were typical of beef cows, and to what extent the Aberdeen Angus udder and the Hereford udders differ from the udders of cows of a dairy breed.

An effort was made to use for comparison the data from dairy cows of approximately the same age as the Herefords, but the only complete and comparable data available were those on two Holsteins that averaged 4 years 7 months of age at time of slaughter as compared with 3 to 3½ years for the Herefords. Moreover, the Aberdeen Angus cow was approximately 12 years of age when slaughtered. Attention is called to this difference in ages as it seems reasonable to suppose it might in some degree affect the size of the animals in the different groups and consequently be reflected in the direct weights and measurements of the body and its parts, and in the comparisons made in them. However, most of the data presented are in the form of ratios of one body measurement to another designed to show body proportions rather than absolute measurements, or are presented as ratios of the size of individual body parts to the body as a whole. A comparison of the data presented in this manner affords an opportunity to study conformation and comparative anatomy more or less irrespective of the size of the animals, and presumably minimizes to a great extent the effect of differences in age.

The Herefords, the Holsteins, and an Aberdeen Angus are compared on the basis of their conformation (ante-mortem data) and their anatomy (post-mortem data); and on the basis of the characteristics of their udders as determined both before and after death. The Aberdeen Angus cow was a show animal (had recently been exhibited at the International Livestock Exposition) and was in a very high condition of flesh, whereas the five Herefords purchased in southern Texas were in comparatively poor flesh. The Holsteins were in moderate flesh.

Holstein cow no. 299 was 4 years 2 months of age and had been dry 22 days when slaughtered. Her production record, made at the age of 3 years 3 months was 14,295 pounds of milk and 463 pounds of butterfat. Holstein cow no. 827 was 4 years 11 months of age, and had been dry 7 months 12 days when slaughtered. Her production record, made at 2 years 7 months of age, was 14,257 pounds of milk and 522 pounds of butterfat. Both Holsteins were milked three times daily while their records were being made. Milking the Holsteins three times instead of twice daily, and the fact that they were slightly more advanced in age at the time the records were made, probably gave them some advantage over the Herefords with respect to abundance of production. However, the difference between the production records of the Herefords and the Holsteins is so great that a marked contrast in producing capacity is emphatically shown. Production records are not available for the Aberdeen Angus cow A–300.

ANTE-MORTEM MEASUREMENTS AND SUPPLEMENTAL DATA

Owing to the lack of facilities at the Iberia Livestock Experiment Farm for slaughtering the Herefords and obtaining the desired post-mortem data, they were weighed on 3 successive days, given the necessary dippings at the Federal dipping station, and shipped to the stockyards at Benning, D. C., where they arrived June 8, 1929, after being en route since June 1.

On June 13 their weights were obtained on the stockyard scales, and photographs were taken of the individual animals. The total weight of the seven animals was 5,900 pounds as compared to 5,903 pounds for the average for 3 days immediately before shipment. Four animals had gained slightly in weight; three had lost. The individual weights are given in table 2.

On June 14, the cows were graded for slaughter purposes as follows:

	Grade
Group 1:	
H–2	Low good.
H–5	Middle medium.
H–6	Low medium.
H–16	Middle good.
Group 2:	
H–1	Top cutter.
H–4	Top medium.
H–17	Low good.

On June 15, at the Benning stockyards, ante-mortem measurements, designed to translate conformation into numerical values suitable for analysis, were made of each of the seven animals according to the plan regularly followed by the Bureau of Dairy Industry in recording and studying growth and conformation. Observations on the development and condition of the udder of each of the seven cows were made on the same day. Live weights, taken at about 2 p. m. on June 15, totaled 5,780 pounds. Individual weights are shown in table 2.

A comparison of the conformation of animals representing such marked differences in type as animals of dairy and beef breeds is difficult to show, except as it can be shown through body proportions, or through the relation of each of the different measurements to one or more measurements selected as most nearly representing the skeletal size of the animal. Bush-Brown (*2*), a sculptor, Washington, D. C., emphasized the necessity for—

an accurate and reliable method of measuring the bodies of individuals, which will make full allowance for variations in size, so that not only large and small individuals of the same breed may become comparable, but also individuals belonging to different breeds, and even the same individual at different stages of growth.

Such a method must clearly be based upon a unit common to all individuals, and upon a comparison of proportions rather than upon absolute measurements. In studying the conformation of the horse, Bush-Brown used as a basis the height at withers which, regardless of the size of the animal, determines the unit of measure to be used for that animal. The proportions obtained from measurements made on this basis enable an artist or a sculptor to reproduce with exactness a painting or a model of any size.

A number of research workers who have studied cattle conformation have indicated that height at withers is a fairly satisfactory measure of skeletal size. According to the views of Van den Bosch, late chief inspector of the Netherlands Cattle Society, a dairy cow approaches perfection as her different body measurements approach definite proportions to height at withers. He uses height at withers as a basis for determining body proportions in evaluating the degree of perfection in the form of a dairy cow. Eckles and Swett (*3, p. 9*) state that—

> growth of the animal, as far as the skeleton is concerned can be determined reasonably well by a few measurements, and sufficiently well for most purposes by one measurement alone.

The one selected was height at withers. Brody (*1, p. 1*) states that—

> since height at withers in cattle is but slightly influenced by environmental conditions, therefore, at a given age the numerical value of this measurement is practically a genetic index of the size of the animal.

More recently Lush and Copeland (*6, p. 49*) stated:

> For most purposes a very few measurements considered in relation to each other or in relation to weight seem as much as would be really useful in contributing to the general picture of the animal and of the changes which occurred in it. Thus height over withers or over hips would certainly be included among those measurements least influenced by plane of nutrition, and therefore especially apt to be illuminating when considered in relation to weight.

Other workers, studying nutrition, growth, and other matters relating to size in cattle, have based their experiments either on skeletal size alone or on skeletal size and live weight—skeletal size being measured by height at withers.

Some students of animal conformation subscribe to the idea that length of head rather than height at withers is the fundamental unit of measurement to which every other body measurement bears a definite ratio in the animal having the desirable form. Pontius (*7, p. 110*) studied the relation between length of head and each of 10 different body measurements for a group of dairy cows having good conformation and good producing capacity. His correlations were not highly significant. According to Gulliver (*5, p. 69*), both Megargee and Kawamura, who are well known as animal painter and animal sculptor respectively, have for years used a system of measurement and proportion based on length of head.

The ante-mortem measurements of the seven Herefords, together with corresponding measurements of the two Holstein cows and the Aberdeen Angus cow, whose udders are compared with those of the Herefords later in this paper are given in table 5.

TABLE 5.—*Ante-mortem data on the animals used in the experiments*

Item	Herefords								Holsteins			Aberdeen Angus
	Group 1 (dairy management)				Group 2 (beef management)			Average	No. 299	No. 827	Average	A-300
	H-2	H-5	H-6	H-16	H-1	H-4	H-17					
Age	(1)	(1)	(1)	(1)	(1)	(1)	(1)		(2)	(3)		(4)
Condition of flesh—grade	6	6	6	7	4	7	8		5	7		9
Time since calving—mos-days	8-14	8-0	8-23	11-19			12-3		11-14	14-7		32-0
Thickness of hide:												
At thirteenth rib—centimeters	1.04	0.97	1.34	1.11	1.01	1.18	0.88		1.00	1.04		1.32
At rear of udder—do	0.63	0.52	0.62	0.60	0.50	0.65	0.55		0.45	0.71		0.56
Diameter of trachea—do	5.39	5.10	4.97		4.34	5.14						
Live weight:												
Actual weight—pounds	780	770	780	955	590	825	1,080	826	1,400	1,375	1,388	1,565
Ratio to height at withers	6.63	6.82	6.69	8.16	5.33	6.79	8.89	7.04	9.80	10.27	10.04	12.81
Ratio to length of head		17.40	18.25	20.21	13.72	18.44	22.38	18.40	26.17	25.46	25.82	33.48
Height at withers:												
Actual measurement—centimeters	117.67	112.92	116.58	117.00	110.75	121.50	121.50	116.85	142.92	133.83	138.38	122.13
Ratio to height at withers	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Ratio to length of head		2.55	2.73	2.48	2.58	2.72	2.52	2.60	2.67	2.48	2.58	2.61
Height at hips:												
Actual measurement—centimeters	118.92	116.08	115.58	117.33	112.75	119.50	124.58	117.82	145.33	135.00	140.17	121.71
Ratio to height at withers	1.01	1.03	0.99	1.00	1.02	0.98	1.03	1.01	1.02	1.01	1.02	1.00
Ratio to length of head		2.62	2.70	2.48	2.62	2.67	2.58	2.61	2.72	2.50	2.61	2.60
Height at pinbones:												
Actual measurement—centimeters	114.33	112.50	112.25	113.88	108.75	116.75	116.58	113.58	132.83	131.83	132.33	119.25
Ratio to height at withers	0.97	1.00	0.96	0.97	0.98	0.96	0.96	0.97	0.93	0.99	0.96	0.98
Ratio to length of head		2.54	2.63	2.41	2.53	2.61	2.42	2.52	2.48	2.44	2.46	2.55
Height of back at first lumbar vertebra:												
Actual measurement—centimeters	117.25	115.25	115.00	116.42	111.25	120.00	120.42	116.51	144.25	131.58	137.92	124.75
Ratio to height at withers	1.00	1.02	0.99	1.00	1.00	0.99	0.99	1.00	1.01	0.98	1.00	1.02
Ratio to length of head		2.60	2.69	2.46	2.59	2.68	2.50	2.59	2.70	2.44	2.57	2.57
Depth of fore chest:												
Actual measurement—centimeters	63.38	62.88	62.88	65.13	58.25	63.13	64.75	62.91	77.25	74.00	75.63	75.00
Ratio to height at withers	0.54	0.56	0.54	0.56	0.53	0.52	0.53	0.54	0.54	0.55	0.55	0.61
Ratio to length of head		1.42	1.47	1.38	1.35	1.41	1.34	1.40	1.44	1.37	1.41	1.60
Depth of rear chest:												
Actual measurement—centimeters	62.00	61.75	61.63	64.50	57.63	62.63	65.50	62.23	78.88	73.50	76.19	78.25
Ratio to height at withers	0.53	0.55	0.53	0.55	0.52	0.52	0.54	0.53	0.55	0.55	0.55	0.64
Ratio to length of head		1.40	1.44	1.37	1.34	1.40	1.36	1.39	1.47	1.36	1.42	1.67
Depth of paunch:												
Actual measurement—centimeters	62.88	62.38	62.50	65.00	56.63	63.88	68.13	63.06	80.13	74.17	77.15	80.25
Ratio to height at withers	0.53	0.55	0.54	0.56	0.51	0.53	0.56	0.54	0.56	0.55	0.56	0.66
Ratio to length of head		1.41	1.46	1.38	1.32	1.43	1.41	1.40	1.50	1.37	1.44	1.72

Length, withers to hips (standard):												
Actual measurement centimeters	81.50	83.50	81.00	84.50	82.00	81.50	87.00	83.00	93.50	98.00	95.75	86.25
Ratio to height at withers	0.69	0.74	0.69	0.72	0.74	0.67	0.72	0.71	0.65	0.73	0.69	0.71
Ratio to length of head		1.89	1.89	1.79	1.91	1.82	1.80	1.85	1.75	1.81	1.78	1.84
Length, hips to pinbones (standard):												
Actual measurement centimeters	40.50	39.50	41.00	44.25	37.50	39.75	44.00	40.93	48.50	47.50	48.00	45.88
Ratio to height at withers	0.34	0.35	0.35	0.38	0.34	0.33	0.36	0.35	0.34	0.35	0.35	0.38
Ratio to length of head		0.89	0.96	0.94	0.87	0.89	0.91	0.91	0.91	0.88	0.90	0.98
Total length, withers to pinbones (standard):												
Actual measurement centimeters	122.00	123.00	122.00	128.75	119.50	121.25	131.00	123.93	142.00	145.50	143.75	132.13
Ratio to height at withers	1.04	1.09	1.05	1.10	1.08	1.00	1.08	1.06	0.99	1.09	1.04	1.08
Ratio to length of head		2.78	2.85	2.72	2.78	2.71	2.72	2.76	2.65	2.69	2.67	2.83
Length of loin:												
Actual measurement centimeters	35.00	35.75	35.25	36.00	35.00	37.25	37.25	35.93	42.00	37.75	39.88	
Ratio to height at withers	0.30	0.32	0.30	0.31	0.32	0.31	0.31	0.31	0.29	0.28	0.29	
Ratio to length of head		0.81	0.82	0.76	0.81	0.83	0.77	0.80	0.79	0.70	.075	
Length of rump:												
Actual measurement centimeters	35.00	34.00	35.00	39.00	33.00	35.00	37.00	35.43	42.00	39.00	40.50	41.50
Ratio to height at withers	0.30	0.30	0.30	0.33	0.30	0.29	0.30	0.30	0.29	0.29	0.29	0.34
Ratio to length of head		0.77	0.82	0.83	0.77	0.78	0.77	0.79	0.79	0.72	0.76	0.89
Length, point of shoulder to pinbones:												
Actual measurement centimeters	142.13	139.00	140.75	148.13	139.63	144.25	148.88	143.25	161.00	166.63	163.82	
Ratio to height at withers	1.21	1.23	1.21	1.27	1.26	1.19	1.23	1.23	1.13	1.25	1.19	
Ratio to length of head		3.14	3.29	3.14	3.25	3.22	3.09	3.19	3.01	3.09	3.05	
Length of head (caliper):												
Actual measurement centimeters		44.25	42.75	47.25	43.00	44.75	48.25	[5] 45.04	53.50	54.00	53.75	46.75
Ratio to height at withers		0.39	0.37	0.40	0.39	0.37	0.40	[5] 0.39	0.37	0.40	0.39	0.38
Ratio to length of head		1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Width of fore chest:												
Actual measurement centimeters	34.42	34.25	36.08	41.75	33.42	34.50	43.33	36.82	54.25	49.83	52.04	61.00
Ratio to height at withers	0.29	0.30	0.31	0.36	0.30	0.28	0.36	0.31	0.38	0.37	0.38	0.50
Ratio to length of head		0.77	0.84	0.88	0.78	0.77	0.90	0.82	1.01	0.92	0.97	1.30
Width of rear chest:												
Actual measurement centimeters	48.25	47.92	48.00	55.25	45.42	49.92	61.00	50.82	62.94	64.50	63.72	65.75
Ratio to height at withers	0.41	0.42	0.41	0.47	0.41	0.41	0.50	0.43	.44	0.48	0.46	0.54
Ratio to length of head		1.08	1.12	1.17	1.06	1.12	1.26	1.14	1.18	1.19	1.19	1.41
Width of paunch:												
Actual measurement centimeters	51.58	53.75	53.75	63.83	49.50	58.83	70.75	57.43	65.00	71.08	68.04	73.75
Ratio to height at withers	0.44	0.48	0.46	0.55	0.45	0.48	0.58	0.49	0.45	0.53	0.49	0.60
Ratio to length of head		1.21	1.26	1.35	1.15	1.31	1.47	1.29	1.21	1.32	1.27	1.58
Width of hips:												
Actual measurement centimeters	48.00	46.25	47.25	50.00	45.00	47.50	52.50	48.07	55.75	55.75	55.75	65.50
Ratio to height at withers	0.41	0.41	0.41	0.43	0.41	0.39	0.43	0.41	0.39	0.42	0.41	0.54
Ratio to length of head		1.05	1.11	1.06	1.05	1.06	1.09	1.07	1.04	1.03	1.04	1.40
Width of thurls:												
Actual measurement centimeters	41.00	41.75	46.00	45.50	39.50	43.50	45.00	43.18	54.00	50.75	52.38	52.38
Ratio to height at withers	0.35	0.37	0.39	0.39	0.36	0.36	0.37	0.37	0.38	0.38	0.38	0.43
Ratio to length of head		0.94	1.08	0.96	0.92	0.97	0.93	0.97	1.01	0.94	0.98	1.12

[1] 3 to 3½ years. [2] 4 years 2 months. [3] 4 years 11 months. [4] About 12 years. [5] Based on 6 animals.

TABLE 5.—*Ante-mortem data on the animals used in the experiments*—Continued

Item	Herefords								Holsteins			Aberdeen Angus
	Group 1 (dairy management)				Group 2 (beef management)			Average	No. 299	No. 827	Average	A-300
	H-2	H-5	H-6	H-16	H-1	H-4	H-17					
Width of pinbones:												
Actual measurement.....centimeters	27.50	29.50	32.50	31.00	24.50	29.50	31.00	29.36	39.50	37.50	38.50	34.50
Ratio to height at withers	0.23	0.26	0.28	0.26	0.22	0.24	0.26	0.25	0.28	0.28	0.28	0.28
Ratio to length of head	.....	0.67	0.76	0.66	0.57	0.66	0.64	0.66	0.74	0.69	0.72	0.74
Width of loin:												
Actual measurement.....centimeters	28.25	27.75	29.75	31.50	27.75	29.50	32.25	29.54	35.00	41.25	38.13	.....
Ratio to height at withers	0.24	0.25	0.26	0.27	0.25	0.24	0.27	0.25	0.24	0.31	0.28	.....
Ratio to length of head	.....	0.63	0.70	0.67	0.65	0.66	0.67	0.66	0.65	0.76	0.71	.....
Width of forehead (caliper):												
Actual measurement.....centimeters	.....	16.75	16.00	16.75	17.25	18.00	17.50	17.04	17.75	19.00	18.38	17.38
Ratio to height at withers	.....	0.15	0.14	0.14	0.16	0.15	0.14	0.15	0.12	0.14	0.13	0.14
Ratio to length of head	.....	0.38	0.37	0.35	0.40	0.40	0.36	0.38	0.33	0.35	0.34	0.37
Width across eyes:												
Actual measurement.....centimeters	.....	20.00	20.25	21.50	20.00	20.75	21.50	20.67	.....	23.25	[6] 23.25	.....
Ratio to height at withers	.....	0.18	0.17	0.18	0.18	0.17	0.18	0.18	.....	0.17	[6] 0.17	.....
Ratio to length of head	.....	0.45	0.47	0.46	0.47	0.46	0.45	0.46	.....	0.43	0.43	.....
Circumference of fore chest:												
Actual measurement.....centimeters	165.50	163.50	164.00	175.00	151.00	165.00	179.00	166.14	214.00	200.00	207.00	230.00
Ratio to height at withers	1.41	1.45	1.41	1.50	1.36	1.36	1.47	1.42	1.50	1.49	1.50	1.88
Ratio to length of head	.....	3.69	3.84	3.70	3.51	3.69	3.71	3.69	4.00	3.70	3.85	4.92
Circumference of rear chest:												
Actual measurement.....centimeters	182.00	177.00	177.00	197.50	167.50	182.00	211.00	184.86	231.50	224.00	227.75	241.50
Ratio to height at withers	1.55	1.57	1.52	1.69	1.51	1.50	1.74	1.58	1.62	1.67	1.65	1.98
Ratio to length of head	.....	4.00	4.14	4.18	3.90	4.07	4.37	4.11	4.33	4.15	4.24	5.17
Circumference of paunch:												
Actual measurement.....centimeters	189.00	191.00	190.00	209.00	173.00	198.00	226.50	196.64	236.00	238.00	237.00	261.00
Ratio to height at withers	1.61	1.69	1.63	1.79	1.56	1.63	1.86	1.68	1.65	1.78	1.72	2.14
Ratio to length of head	.....	4.32	4.44	4.42	4.02	4.42	4.69	4.39	4.41	4.41	4.41	5.58
Circumference of muzzle:												
Actual measurement.....centimeters	52.00	46.00	45.00	48.00	45.00	44.50	47.50	46.86	52.00	54.00	53.00	47.75
Ratio to height at withers	0.44	0.41	0.39	0.41	0.41	0.37	0.39	0.40	0.36	0.40	0.38	0.39
Ratio to length of head	.....	1.04	1.05	1.02	1.05	0.99	0.98	1.02	0.97	1.00	0.99	1.02
Circumference of shinbone:												
Actual measurement.....centimeters	17.00	17.75	17.50	18.00	16.50	17.75	17.75	17.46	18.75	19.25	19.00	18.75
Ratio to height at withers	0.14	0.16	0.15	0.15	0.15	0.15	0.15	0.15	0.13	0.14	0.14	0.15
Ratio to length of head	.....	0.40	0.41	0.38	0.38	0.40	0.37	0.39	0.35	0.36	0.36	0.40

[6] Based on 1 animal.

BREED DIFFERENCES IN UNITS OF MEASUREMENT AND IN RATIOS TO HEIGHT AT WITHERS AND TO LENGTH OF HEAD

When expressed in actual units, the measurements of the Herefords differed considerably from each other, and as a group differed markedly from the Holsteins and from the Aberdeen Angus. Undoubtedly, condition of fleshing and stage of gestation had a considerable effect on the measurements of individual animals, as H–1 was in comparatively poor flesh, H–17 was in very good flesh and nearly due to freshen, and A–300 was in a very high condition of flesh.

The ratio of each body measurement to height at withers and to length of head, which, as stated, have been used by several investigators as basic measurements, have been determined to make possible a study of the comparative proportions of each animal. Table 5 shows the body measurements for the different items expressed in actual units, and the ratios of the body measurements to the height at withers and to the length of head. Thickness of hide and diameter of trachea are not considered as body measurements, consequently they are not included in the discussion of conformation.

Without exception the average for live weight, and for each body measurement, expressed in actual units, was greater for the Holsteins than for the Herefords, but the relation of actual measurements of the Aberdeen Angus to those of the other breeds is not consistent. The probable effect of age differences on these absolute measurements has been mentioned previously. Twenty-four different body measurements are compared for each of the breeds. The magnitude of the measurement for the Aberdeen Angus is intermediate between the averages for the Herefords and the Holsteins for 13 of these items, 8 of which are indicative of "scale." The width of thurls of the Aberdeen Angus is equal to the corresponding average for Holsteins but distinctly greater than the Hereford average. The measurements for the remaining 10 items, which include the depths of rear chest and of paunch; length of rump; width of hips; and the widths and circumferences of fore chest, rear chest, and paunch, are greater for the Aberdeen Angus than for either the Herefords or the Holsteins. Undoubtedly most of these 10 measurements, as well as the width of thurls, were affected to a very considerable extent by the excessive fleshing in the Aberdeen Angus.

PERCENTAGE DIFFERENCES, BY BREEDS, IN UNITS OF MEASUREMENT AND IN RATIOS

In comparing breed averages on the basis either of actual units of measurement or of ratios, some differences which at first appeared small were found to be of considerable importance if they represented small measurements or low ratios. Some differences that appeared relatively large were of comparatively little significance if they represented large measurements or high ratios. For example, in comparing the averages for Holsteins with those for Herefords (table 5), a difference of 21.53 centimeters in height at withers represents a percentage of 18.4, whereas a difference of only 8.59 centimeters in width of loin is equivalent to 29.1 percent. For this reason the differences in breed averages were reduced to a percentage basis. To determine the percentage to which the Holsteins differed from the Herefords for any given item the difference between the averages for the two breeds for that item was divided by the average for the

TABLE 6.—*Comparative conformation of the Herefords, Holsteins, and Aberdeen Angus*

Item	Actual units			Ratios based on heights at withers			Ratios based on length of head		
	Relation of Holsteins to Herefords	Relation of Aberdeen Angus to Herefords	Relation of Aberdeen Angus to Holsteins	Relation of Holsteins to Herefords	Relation of Aberdeen Angus to Herefords	Relation of Aberdeen Angus to Holsteins	Relation of Holsteins to Herefords	Relation of Aberdeen Angus to Herefords	Relation of Aberdeen Angus to Holsteins
	Percent	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
Live weight	+68.04	+89.47	+12.75	+42.61	+81.96	+27.59	+40.33	+81.96	+29.67
Height at withers	+18.43	+4.52	−11.74				−.77	+.38	+1.16
Height at hips	+18.97	+3.30	−13.17	+.99	−.99	−1.96	±.00	−.38	−.38
Height at pinbones	+16.51	+4.99	−9.88	−1.03	+1.03	+2.08	−2.38	+1.19	+3.66
Height of back at first lumbar vertebra	+18.38	+7.07	−9.55	±.00	+2.00	+2.00	−.77	+3.09	+3.89
Depth of fore chest	+20.22	+19.22	−.83	+1.85	+12.96	+10.91	+.71	+14.29	+13.48
Depth of rear chest	+22.43	+25.74	+2.70	+3.77	+20.75	+16.36	+2.16	+20.14	+17.61
Depth of paunch	+22.34	+27.26	+4.02	+3.70	+22.22	+17.86	+2.86	+22.86	+19.44
Length, withers to hips (standard)	+15.36	+3.92	−9.92	−2.82	±.00	+2.90	−3.78	−.54	+3.37
Length, hips to pinbones (standard)	+17.27	+12.09	−4.42	±.00	+8.57	+8.57	−1.10	+7.69	+8.89
Total length, withers to pinbones	+15.99	+6.62	−8.08	−1.89	+1.89	+3.85	−3.26	+2.54	+5.99
Length of loin	+10.99			−6.45			−6.25		
Length of rump	+14.31	+17.13	+2.47	−3.33	+13.33	+17.24	−3.80	+12.66	+17.11
Length, point of shoulder to pinbones	+14.36			−3.25			−4.39		
Length of head (caliper)	+19.34	+3.80	−13.02	±.00	−2.56	−2.56			
Width of fore chest	+41.34	+65.67	+17.22	+22.58	+61.29	+31.58	+18.29	+58.54	+34.02
Width of rear chest	+25.38	+29.38	+3.19	+6.98	+25.58	+17.39	+4.39	+23.68	+18.49
Width of paunch	+18.47	+28.42	+8.39	±.00	+22.45	+22.45	−1.55	+22.48	+24.41
Width of hips	+15.98	+36.26	+17.49	±.00	+31.71	+31.71	−2.80	+30.84	+34.62
Width of thurls	+21.31	+21.31	±.00	+2.70	+16.22	+13.16	+1.03	+15.46	+14.29
Width of pinbones	+31.13	+17.51	−10.39	+12.00	+12.00	±.00	+9.09	+12.12	+2.78
Width of loin	+29.08			+12.00			+7.58		
Width of forehead (caliper)	+7.86	+2.00	−5.44	−13.33	−6.67	+7.69	−10.53	−2.63	+8.82
Width across eyes	+12.48			−5.56			−6.52		
Circumference of fore chest	+24.59	+38.44	+11.11	+5.63	+32.39	+25.33	+4.34	+33.33	+27.79
Circumference of rear chest	+23.20	+30.64	+6.04	+4.43	+25.32	+20.00	+3.16	+25.79	+21.93
Circumference of paunch	+20.52	+32.73	+10.13	+2.38	+27.38	+24.42	+.46	+27.11	+26.53
Circumference of muzzle	+13.10	+1.90	−9.91	−5.00	−2.50	+2.63	−2.94	±.00	+3.03
Circumference of shinbone	+8.82	+7.39	−1.32	−6.67	±.00	+7.14	−7.69	+2.56	+11.11

Herefords and multiplied by 100. If the Holstein average was higher than the Hereford average, the percentage value was prefixed with a plus sign; if lower it was prefixed with a minus sign. In the same manner the data for the Aberdeen Angus were compared first with the Herefords and then with the Holsteins. The percentages showing the extent to which the breeds differ are given in table 6.

Since all the weights and measurements are greater for Holsteins than for Herefords, the percentages showing the degree of difference are all plus. The greatest difference, which is for live weight, shows that the Holsteins averaged 68.04 percent heavier than the Herefords. Among the measurements, the greatest difference was in width of fore chest (41.34 percent), and the next in width of pinbones (31.13 percent). The greatest differences appear to be associated with fullness of barrel and the smallest with skeletal measurements and "scale."

The Aberdeen Angus differs from the Herefords most in live weight, widths and circumferences, and least in skeletal measurements and "scale."

The Aberdeen Angus is higher than the Holsteins in weight, lower than the Holsteins for 13 of the measurements, higher for 10, and the same for 1. The measurements having the greatest minus difference are height at withers and hips, length of head, and width of pinbones. Those having the greatest plus difference are width of fore chest and hips and circumference of fore chest and paunch.

The ratios based on height at withers indicate that the Holsteins were very similar to the Herefords in body proportions with only a few of the items differing greatly, the larger differences being confined almost entirely to measurements of width. The Aberdeen Angus differed most from both the Herefords and the Holsteins in width of hips, depth of paunch, and in widths and circumferences of barrel, showing the combined effect on body proportions of a high condition of flesh in a low-set animal.

The comparison of the ratios based on length of head emphasizes the similarity in the proportions of the Herefords and Holsteins for nearly all items; it shows that the Aberdeen Angus differed only slightly from the Herefords and the Holsteins in the items representing "scale", but that the Aberdeen Angus differed greatly from both Herefords and Holsteins in many of the widths and in all of the circumferences of the barrel. In view of these results it is noteworthy that the Herefords and the Aberdeen Angus, though differing greatly in size and in many body measurements, were nearly the same in length of head, and that the Holsteins greatly exceeded them in this measurement.

Although marked differences existed in the live weight and in many of the body dimensions of the breeds, a similarity is shown in the body proportions of the Holsteins and the Herefords when the ante-mortem data are compared on the basis of ratios based either on height at withers or on length of head. Also the ratios for measurements of height and length, which are indicative of "scale", indicate that the three breeds are very similar. The outstanding differences in body proportions are for those measurements that are most affected by deposition of fat. This confirms conclusions previously expressed (*8*) that cows of the different breeds are generally similar in skeletal structure, and that difference in type resulting from breeding and selection is due primarily to degree of fleshing.

TABLE 7.—*Supplemental ante-mortem data on the animals used in the experiments*

Item	Herefords								Holsteins			Aberdeen Angus
	Group 1 (dairy management)				Group 2 (beef management)			Average				
	H-2	H-5	H-6	H-16	H-1	H-4	H-17		No. 299	No. 827	Average	A-300
Contour area of fore chest:												
Total area—square centimeters	1,692	1,657	1,742	2,053	1,443	1,721	2,122	1,776	3,112	2,747	2,930	3,960
Portion above vertical midpoint—percent	52.6	53.3	51.3	51.1	53.6	52.2	50.5	52.1	48.6	49.6	49.1	51.1
Portion below vertical midpoint—do	47.4	46.7	48.7	48.9	46.4	47.8	49.5	47.9	51.4	50.4	50.9	48.9
Total area divided by depth of fore chest—ratio	26.7	26.4	27.7	31.5	24.8	27.3	32.8	28.2	40.3	37.1	38.7	52.8
Total area divided by width of fore chest—do	49.2	48.4	48.3	49.2	43.2	49.9	49.0	48.2	57.4	55.1	56.3	64.9
Total area divided by circumference of fore chest—ratio	10.2	10.1	10.6	11.7	9.6	10.4	11.9	10.6	14.5	13.7	14.1	17.2
Contour area of paunch:												
Total area—square centimeters	2,602	2,634	2,663	3,233	2,145	2,891	3,841	2,858	3,910	4,127	4,019	4,916
Portion above vertical midpoint—percent	49.0	49.1	48.9	47.9	48.9	48.1	48.3	48.6	44.8	49.6	47.2	49.3
Portion below vertical midpoint—do	51.0	50.9	51.1	52.1	51.1	52.0	51.7	51.4	55.3	50.4	52.9	50.8
Total area divided by depth of paunch—ratio	41.4	42.2	42.6	49.7	37.9	45.3	56.4	45.1	48.8	55.6	52.2	61.3
Total area divided by width of paunch—do	50.5	49.0	49.5	50.7	43.3	49.1	54.3	49.5	60 2	58.1	59.2	66.7
Total area divided by circumference of paunch—ratio	13.8	13.8	14.0	15.5	12.4	14.6	17.0	14.4	16.6	17.3	17.0	18.8
Body surface area—square centimeters	41,323	40,816	41,224	46,285	36,337	42,067	48,838	42,413	57,742	58,132	57,937	57,030
Volume of barrel—cubic centimeters	174,689	174,076	179,022	233,300	152,020	191,149	263,529	195,398	346,962	344,800	345,881	356,447
Angle of rump (clinometer)—degrees—minutes	−7°55′	−7°10′	−7°27′	−5°24′	−8°26′	−6°14′	−13°18′	−7°59′	−15°48′	−4°54′	−10°21′	−3°52′
Thoracic index	1.841	1.836	1.743	1.560	1.743	1.830	1.494	1.721	1.424	1.485	1.455	1.230
Abdominal index	1.219	1.161	1.163	1.018	1.144	1.086	0.963	1.108	1.233	1.043	1.138	1.088
Legginess	0.461	0.443	0.461	0.443	0.474	0.480	0.467	0.461	0.459	0.447	0.453	0.386
Wedge shape:												
Based on differences in measurements—												
Depth of paunch minus depth of fore chest—centimeters	−0.50	−0.50	−0.38	−0.13	−1.62	0.75	3.38	0.14	2.88	0.17	1.53	5.25
Width of paunch minus width of fore chest—centimeters	17.16	19.50	17.67	22.08	16.08	24.33	27.42	20.61	10.75	21.25	16.00	12.75
Circumference of paunch minus circumference of fore chest—centimeters	23.50	27.50	26.00	34.00	22.00	33.00	47.50	30.50	22.00	38.00	30.00	31.00
Contour area of paunch minus contour area of fore chest—square centimeters	910	977	921	1,180	703	1,170	1,719	1,083	798	1,380	1,089	956
Based on ratios of measurements—												
Depth of paunch divided by depth of fore chest—ratio	0.992	0.992	0.994	0.998	0.972	1.012	1.052	1.002	1.037	1.002	1.020	1.070
Width of paunch divided by width of fore chest—ratio	1.499	1.569	1.490	1.529	1.481	1.705	1.633	1.558	1.198	1.426	1.312	1.209
Circumference of paunch divided by circumference of fore chest—ratio	1.142	1.168	1.159	1.194	1.146	1.200	1.265	1.182	1.103	1.190	1.147	1.135
Contour area of paunch divided by contour area of fore chest—ratio	1.538	1.590	1.529	1.575	1.486	1.680	1.810	1.601	1.256	1.502	1.379	1.241

In addition to the ante-mortem measurements given in table 5 a number of additional values have been derived to show more completely the differences in the conformation of the Herefords, the Holsteins, and the Aberdeen Angus. The methods employed in obtaining the contours, body surface area, volume of barrel, angle of rump, thoracic and abdominal indexes, legginess, and wedge shape are explained in detail in a previous publication (*9*). These supplemental ante-mortem data are given in table 7.

Attention is again called to the fact that the Holsteins and the Aberdeen Angus were older than the Herefords at the time of slaughter and that this greater maturity probably had an effect on the absolute values representing total area of fore chest and of paunch, body surface area, and volume of barrel. A marked progressive increase is shown from Hereford to Holstein to Aberdeen Angus in the average values for total contour area of both fore chest and paunch. In the fore-chest contours, slightly more than half the area is above the vertical midpoint for Herefords and for the Aberdeen Angus, whereas very slightly more than half was below for the Holsteins. In the paunch slightly more than half of the total area was below the vertical midpoint for all three groups. In both fore chest and paunch, the values obtained by dividing the total contour area by the corresponding depth, width, and circumference, progress with considerable regularity from Herefords to Holsteins to Aberdeen Angus. However, the increase is less marked from Holsteins to Aberdeen Angus in the case of circumference of paunch, than for the others.

The body surface area is much less for the Herefords than for the Holsteins, but almost the same for the Aberdeen Angus as for the Holsteins. A similar relationship of values exists for the estimated volume of barrel. Though perhaps not highly significant, the angle of rump was highest for the Holsteins and lowest for the Aberdeen Angus. The thoracic index decreases with considerable regularity from Herefords to Holsteins to Aberdeen Angus, signifying that the Holsteins were intermediate and that the Aberdeen Angus was the highest in proportion of width to depth of fore chest. Values for abdominal index are nearly the same for all three breeds. The legginess was almost the same for the Holsteins as for the Herefords, but distinctly less for the Aberdeen Angus.

On the basis of actual measurements, the Herefords had almost no vertical wedge shape, as determined by subtracting depth of fore chest from depth of paunch; the Holsteins had more than 10 times as much as the Herefords; and the Aberdeen Angus had more than 3 times as much as the Holsteins. In width, the wedge shape decreased markedly from Herefords to Holsteins to Aberdeen Angus, showing that the Herefords were narrowest in the chest as compared with the width of paunch. In circumference the wedge shape was almost the same for all three breeds; and on the basis of the contour areas the wedge shape of the Herefords and Holsteins was almost identical, but that of the Aberdeen Angus was considerably less.

Another method of showing wedge shape is to divide the paunch measurement by the corresponding fore-chest measurement. The ratios for depth obtained in this manner increase somewhat from Herefords to Holsteins and to a greater extent from Holsteins to Aberdeen Angus. On the other hand, the corresponding ratios for widths decrease. Ratios for circumferences are highest for the

Herefords, but those for Holsteins and for Aberdeen Angus are nearly the same. For contour areas a decrease in ratios is shown from Herefords to Holsteins to Aberdeen Angus. The ratios show practically the same thing as the differences in measurements, indicating that the Herefords were relatively shallow in the paunch, whereas the Holsteins had somewhat greater and the Aberdeen Angus much greater relative paunch depth; that in width the Herefords were relatively narrow-chested, whereas the Holsteins had greater and the Aberdeen Angus still greater relative width of fore chest; that in circumference the relation of paunch to fore chest was more nearly the same for all breeds and that in contour area the relation of paunch to fore chest was lower for the Aberdeen Angus than for the Holsteins, and lower for both than for the Herefords. The data in table 7 emphasize again the effect of fleshing on the body proportions of the animal.

POST-MORTEM ANATOMICAL DATA

The seven Herefords were slaughtered during the period of June 17 to 21, 1929, and post-mortem data were obtained in accordance with the general plan adopted by the Bureau of Dairy Industry for studying the relation between the conformation and anatomy of the dairy cow and her milk- and butterfat-producing capacity. The post-mortem data for the Herefords, the Holsteins, and the Aberdeen Angus are presented in table 8. The live weights differ somewhat from those shown in the ante-mortem data as they were taken immediately before slaughter, whereas the ante-mortem data sometimes were obtained several days earlier. Empty body weight is used to represent the net weight of the total animal structure. It is determined by subtracting the total weight of the contents of the stomachs and intestines from the live weight. It is more significant than live weight as it eliminates variations in weight due to the "fill" of the animal. The other items are self-explanatory.

In table 8, data are given for each animal in the units of weight or measurement as taken, and show the differences existing between individuals or breeds in the size of the animal or its organs and body parts. Here, again, the authors call attention to the possible effect of age differences on these absolute values and on comparisons based upon them. The values designated as "units per 100 pounds of empty body weight", show the relation of each organ and body part to the weight of total animal structure.

The actual values for Holsteins are greater than those for Herefords in all but 3 of the 67 items compared. The actual values for the Aberdeen Angus are higher than those for the Herefords in all but 4 of the 34 items compared, but greater than those for the Holsteins in only 11 of the 34. When compared on the basis of the values showing size of organ or body part in relation to empty body weight, the Herefords exceed the Holsteins in 53 of the 66 items compared, and the Herefords exceed the Aberdeen Angus in 26 of the 33 items compared. The Holsteins exceed the Aberdeen Angus in 27 of the 33 items compared. To obtain a better idea of the degree of difference between the breeds, both the actual units and the values based on empty body weight are compared, as in the case of the ante-mortem data, by reducing the differences to a percentage basis. The percentages for the different comparisons for each item are shown in table 9.

TABLE 8.—*Post-mortem data on the animals used in the experiments*

Item	Herefords								Holsteins			Aberdeen Angus
	Group 1 (dairy management)				Group 2 (beef management)			Average	No. 299	No. 827	Average	A-300
	H-2	H-5	H-6	H-16	H-1	H-4	H-17					
Live weight:												
Actual weight, pounds	795	735	790	935	570	805	1,095	818	1,420	1,338	1,379	1,585
Units per 100 pounds empty body weight	115.64	123.20	127.47	117.07	125.56	121.36	117.37	121.10	114.81	113.78	114.30	107.36
Empty body weight:												
Actual weight, pounds	687.45	596.60	619.75	798.65	453.95	663.30	932.95	678.95	1,236.85	1,175.94	1,206.40	1,476.36
Weight of blood:												
Actual weight, do	30.25	23.75	24.00	27.25	----------	24.75	36.75	27.79	62.00	48.75	55.38	----------
Units per 100 pounds empty body weight	4.40	3.98	3.87	3.41	----------	3.73	3.94	3.89	5.01	4.15	4.58	----------
Weight of thyroid:												
Actual weight, grams	21.0	11.5	12.5	19.0	12.2	----------	----------	15.24	39.0	40.4	39.70	57.50
Units per 100 pounds empty body weight	3.05	1.93	2.02	2.38	2.69	----------	----------	2.41	3.15	3.44	3.30	3.89
Weight of brain:												
Actual weight, grams	406.0	341.0	385.0	353.0	325.5	326.5	362.4	357.06	454.0	507.0	480.50	----------
Units per 100 pounds empty body weight	59.06	57.16	62.12	44.20	71.70	49.22	38.84	54.61	36.71	43.11	39.91	----------
Weight of pituitary body:												
Actual weight, grams	2.50	2.00	2.00	2.10	1.70	2.50	2.50	2.19	4.20	3.23	3.72	----------
Units per 100 pounds empty body weight	0.36	0.34	0.32	0.26	0.37	0.38	0.27	0.33	0.34	0.27	0.31	----------
Weight of udder (empty):												
Actual weight, pounds	9.95	7.15	8.35	14.50	6.30	6.35	18.00	10.09	30.50	22.00	26.25	44.00
Units per 100 pounds empty body weight	1.45	1.20	1.35	1.82	1.39	0.96	1.93	1.44	2.47	1.87	2.17	2.98
Weight of hide:												
Actual weight, pounds	64.00	66.00	72.00	64.00	54.00	62.00	65.00	63.86	79.75	81.43	80.59	82.50
Units per 100 pounds empty body weight	9.31	11.06	11.62	8.01	11.90	9.35	6.97	9.75	6.45	6.92	6.69	5.59
Weight of genital organs:												
Actual weight, pounds	21.70	3.40	5.75	77.50	1.20	6.15	98.25	30.56	3.60	3.05	3.33	2.95
Units per 100 pounds empty body weight	3.16	0.57	0.93	9.70	0.26	0.93	10.53	3.73	0.29	0.26	0.28	0.20
Weight of ovaries:												
Actual weight, grams	11.0	9.2	15.2	17.0	7.0	11.7	13.5	12.09	37.2	14.1	25.65	34.40
Units per 100 pounds empty body weight	1.60	1.54	2.45	2.13	1.54	1.76	1.45	1.78	3.01	1.20	2.11	2.33
Weight of amniotic fluid:												
Actual weight, grams	2,790	378	959	6,532	----------	255	6,078	2,832	----------	----------	----------	----------
Units per 100 pounds empty body weight	405.85	63.36	154.74	817.88	----------	38.44	651.48	355.29	----------	----------	----------	----------
Weight of fetus:												
Actual weight, grams	3,561	46	192	17,464	----------	236	27,443	8,157	----------	----------	----------	----------
Units per 100 pounds empty body weight	518.00	7.71	30.98	2,186.69	----------	35.58	2,941.53	953.42	----------	----------	----------	----------
Period of gestation, days	157	73	91	223	(1)	(2)	271	----------	(1)	(1)	----------	(1)

[1] Not pregnant.

[2] Period of gestation not known.

TABLE 8.—*Post-mortem data on the animals used in the experiments*—Continued

Item	Herefords								Holsteins			Aberdeen Angus
	Group 1 (dairy management)				Group 2 (beef management)			Average	No. 299	No. 827	Average	A-300
	H-2	H-5	H-6	H-16	H-1	H-4	H-17					
Weight fetal membranes:												
Actual weight, grams	1,134	266	332	3,629		55		1,083.2				
Units per 100 pounds empty body weight	164.96	44.59	53.57	454.39		8.29		145.16				
Weight of liver:												
Actual weight, pounds	9.55	7.00	8.95	7.60	6.05	9.40	10.40	8.42	16.95	13.85	15.40	14.75
Units per 100 pounds empty body weight	1.39	1.17	1.44	0.95	1.33	1.42	1.11	1.26	1.37	1.18	1.28	1.00
Weight of spleen:												
Actual weight, grams	645.0	478.5	533.0	572.0	386.0	500.5	809.0	560.57	1,058.0	632.0	845.00	839.20
Units per 100 pounds empty body weight	93.83	80.20	86.00	71.62	85.03	75.46	86.71	82.69	85.54	53.74	69.64	56.84
Weight of pancreas:												
Actual weight, grams	261.5	160.0	239.5	208.5	146.2		231.7	207.90	582.5	508.5	545.50	371.00
Units per 100 pounds empty body weight	38.04	26.82	38.64	26.11	32.21		24.84	31.11	47.10	43.24	45.17	25.13
Length of small intestine:												
Actual measurement, feet	120.87	113.16	112.50	112.34	93.15	112.67	127.26	113.14	171.54	144.22	157.88	140.22
Units per 100 pounds empty body weight	17.58	18.97	18.15	14.07	20.52	16.99	13.64	17.13	13.87	12.26	13.07	9.50
Length of large intestine:												
Actual measurement, feet	31.98	30.50	29.85	30.60	23.45	26.73	37.06	30.02	45.92	43.16	44.54	41.49
Units per 100 pounds empty body weight	4.65	5.11	4.82	3.83	5.17	4.03	3.97	4.51	3.71	3.67	3.69	2.81
Total length of intestines:												
Actual measurement, feet	152.85	143.66	142.35	142.94	116.60	139.40	164.32	143.16	217.46	187.38	202.42	181.71
Units per 100 pounds empty body weight	22.23	24.08	22.97	17.90	25.69	21.02	17.61	21.64	17.58	15.93	16.76	12.31
Weight of small intestine:												
Actual weight, pounds	11.10	10.35	9.35	9.20	9.50	10.60	12.30	10.34	13.25	9.75	11.50	8.70
Units per 100 pounds empty body weight	1.61	1.73	1.51	1.15	2.09	1.60	1.32	1.57	1.07	0.83	0.95	0.59
Weight of large intestine:												
Actual weight, pounds	10.35	9.30	7.85	10.50	7.10	11.60	12.50	9.89	9.90	8.05	8.98	7.75
Units per 100 pounds empty body weight	1.51	1.56	1.27	1.31	1.56	1.75	1.34	1.47	0.80	0.68	0.74	0.52
Total weight of intestines:												
Actual weight, pounds	21.45	19.65	17.20	19.70	16.60	22.20	24.80	20.23	23.15	17.80	20.48	16.45
Units per 100 pounds empty body weight	3.12	3.29	2.78	2.47	3.66	3.35	2.66	3.05	1.87	1.51	1.69	1.11
Weight of paunch:												
Actual weight, pounds	13.00	11.60	12.70	10.95	8.65	13.20	15.10	12.17	24.85	16.65	20.75	14.95
Units per 100 pounds empty body weight	1.89	1.94	2.05	1.37	1.91	1.99	1.62	1.82	2.01	1.42	1.72	1.01
Weight of reticulum:												
Actual weight, pounds	2.30	1.95	1.75	2.25	1.60	2.05	2.10	2.00	3.70	2.53	3.12	2.85
Units per 100 pounds empty body weight	0.33	0.33	0.28	0.28	0.35	0.31	0.23	0.30	0.30	0.22	0.26	0.19

Weight of omasum:												
Actual weight, pounds	8.45	7.60	7.45	7.85	4.70	6.95	8.40	7.34	13.50	9.33	11.42	8.80
Units per 100 pounds empty body weight	1.23	1.27	1.20	0.98	1.04	1.05	0.90	1.10	1.09	0.79	0.94	0.60
Weight of abomasum:												
Actual weight, pounds	3.55	2.95	4.00	3.85	5.05	3.90	4.45	3.96	4.90	3.28	4.09	4.75
Units per 100 pounds empty body weight	0.52	0.49	0.65	0.48	1.11	0.59	0.48	0.62	0.40	0.28	0.34	0.32
Total weight stomachs:												
Actual weight, pounds	27.30	24.10	25.90	24.90	20.00	26.10	30.05	25.48	46.95	31.79	39.37	31.35
Units per 100 pounds empty body weight	3.97	4.04	4.18	3.12	4.41	3.93	3.22	3.84	3.80	2.70	3.25	2.12
Total weight of abdominal fat:												
Actual weight, pounds	13.30	7.70	8.75	24.55	3.95	9.35	19.65	12.46	46.10	81.10	63.60	87.15
Units per 100 pounds empty body weight	1.93	1.29	1.41	3.07	0.87	1.41	2.11	1.73	3.73	6.90	5.32	5.90
Weight of pluck:												
Actual weight, pounds	----------	11.25	12.45	15.35	9.75	12.70	15.55	12.84	27.55	29.20	28.38	26.50
Units per 100 pounds empty body weight	----------	1.89	2.01	1.92	2.15	1.91	1.67	1.93	2.23	2.48	2.36	1.80
Weight of right lung:												
Actual weight, pounds	2.85	2.70	2.80	3.15	2.60	2.85	2.90	2.84	5.40	4.80	5.10	3.15
Units per 100 pounds empty body weight	0.41	0.45	0.45	0.39	0.57	0.43	0.31	0.43	0.44	0.41	0.43	0.21
Weight left lung:												
Actual weight, pounds	2.20	2.00	2.20	2.50	1.90	2.15	2.25	2.17	4.20	3.30	3.75	2.70
Units per 100 pounds empty body weight	0.32	0.34	0.35	0.31	0.42	0.32	0.24	0.33	0.34	0.28	0.31	0.18
Total weight lungs:												
Actual weight, pounds	5.05	4.70	5.00	5.65	4.50	5.00	5.15	5.01	9.60	8.10	8.85	5.85
Units per 100 pounds empty body weight	0.73	0.79	0.81	0.71	0.99	0.75	0.55	0.76	0.78	0.69	0.74	0.40
Weight heart, auricles attached:												
Actual weight, pounds	2.50	2.25	2.50	2.80	1.95	2.60	3.05	2.52	5.55	4.70	5.13	4.30
Units per 100 pounds empty body weight	0.36	0.38	0.40	0.35	0.43	0.39	0.33	0.38	0.45	0.40	0.43	0.29
Circumference heart (base):												
Actual measurement, centimeters	41.00	----------	40.50	41.00	36.50	40.75	43.00	40.46	51.60	51.80	51.70	51.00
Units per 100 pounds empty body weight	5.96	----------	6.53	5.13	8.04	6.14	4.61	6.07	4.17	4.40	4.29	3.45
Circumference heart (apex 1):												
Actual measurement, centimeters	41.00	----------	42.00	41.00	38.25	41.50	44.00	41.20	54.30	51.35	52.83	48.50
Units per 100 pounds empty body weight	5.96	----------	6.78	5.13	8.43	6.26	4.72	6.21	4.39	4.37	4.38	3.29
Circumference heart (apex 2):												
Actual measurement, centimeters	45.75	----------	48.75	47.00	44.00	48.50	49.00	47.17	63.35	59.15	61.25	55.00
Units per 100 pounds empty body weight	6.66	----------	7.87	5.88	9.69	7.31	5.25	7.11	5.12	5.03	5.08	3.73
Weight heart, auricles removed:												
Actual weight, pounds	2.30	2.00	2.30	2.55	1.75	2.40	2.75	2.29	5.05	4.25	4.65	3.90
Units per 100 pounds empty body weight	0.33	0.34	0.37	0.32	0.39	0.36	0.29	0.34	0.41	0.36	0.39	0.26
Total weight of thoracic fat:												
Actual weight, pounds	2.25	1.90	1.70	3.30	0.45	1.25	3.65	2.07	4.60	11.50	8.05	8.75
Units per 100 pounds empty body weight	0.33	0.32	0.27	0.41	0.10	0.19	0.39	0.29	0.37	0.98	0.68	0.59
Weight of kidneys:												
Actual weight, grams	740.00	626.00	581.70	670.00	547.50	779.00	757.50	671.67	1,313.00	1,094.50	1,203.75	1,746.40
Units per 100 pounds empty body weight	107.64	104.93	93.86	83.89	120.61	117.44	81.19	101.37	106.16	93.07	99.62	118.30
Weight of adrenals:												
Actual weight, grams	16.50	17.50	21.00	14.50	19.70	19.80	20.2	18.46	36.5	24.00	30.25	57.50
Units per 100 pounds empty body weight	2.40	2.93	3.39	1.82	4.34	2.99	2.17	2.86	2.95	2.04	2.50	3.89
Weight of dressed carcass:												
Actual weight, pounds	398.00	378.00	638.00	464.00	274.00	420.00	538.00	408.57	773.00	734.50	753.75	----------
Units per 100 pounds empty body weight	57.90	63.36	82.61	58.10	60.36	63.32	57.67	60.47	62.50	62.46	62.48	----------

TABLE 8.—*Post-mortem data on the animals used in the experiments*—Continued

Item	Herefords								Holsteins			Aberdeen Angus
	Group 1 (dairy management)				Group 2 (beef management)			Average	No. 209	No. 827	Average	A-300
	H-2	H-5	H-6	H-16	H-1	H-4	H-17					
Depth thoracic cavity:												
First thoracic vertebra:												
Actual measurement----------centimeters	16.00	16.00	13.25	15.25	14.00	12.50	17.50	14.93	20.00	17.50	18.75	----------
Units per 100 pounds empty body weight	2.33	2.68	2.14	1.91	3.08	1.88	1.88	2.27	1.62	1.49	1.56	----------
Second thoracic vertebra:												
Actual measurement----------centimeters	22.00	22.25	21.00	22.75	20.00	20.25	24.25	21.79	29.00	20.50	24.75	----------
Units per 100 pounds empty body weight	3.20	3.73	3.39	2.85	4.41	3.05	2.60	3.32	2.34	1.74	2.04	----------
Third thoracic vertebra:												
Actual measurement----------centimeters	28.00	27.25	27.25	28.75	26.00	25.75	29.00	27.43	36.25	30.50	33.38	----------
Units per 100 pounds empty body weight	4.07	4.57	4.40	3.60	5.73	3.88	3.11	4.19	2.93	2.59	2.76	----------
Fourth thoracic vertebra:												
Actual measurement----------centimeters	31.75	33.25	33.00	34.25	31.25	31.25	34.75	32.79	43.75	36.75	40.25	----------
Units per 100 pounds empty body weight	4.62	5.57	5.32	4.29	6.88	4.71	3.72	5.02	3.54	3.13	3.34	----------
Fifth thoracic vertebra:												
Actual measurement----------centimeters	36.50	36.25	36.50	37.25	35.00	36.50	38.00	36.57	47.00	40.75	43.88	----------
Units per 100 pounds empty body weight	5.31	6.08	5.89	4.66	7.71	5.50	4.07	5.60	3.80	3.47	3.64	----------
Sixth thoracic vertebra:												
Actual measurement----------centimeters	39.00	38.75	39.50	39.00	37.75	40.25	39.75	39.14	49.50	44.25	46.88	----------
Units per 100 pounds empty body weight	5.67	6.50	6.37	4.88	8.32	6.07	4.26	6.01	4.00	3.76	3.88	----------
Seventh thoracic vertebra:												
Actual measurement----------centimeters	42.00	41.50	41.50	41.25	39.50	41.75	41.75	41.32	51.75	46.75	49.25	----------
Units per 100 pounds empty body weight	6.11	6.96	6.70	5.16	8.70	6.29	4.48	6.34	4.18	3.98	4.08	----------
Eighth thoracic vertebra:												
Actual measurements----------centimeters	44.00	43.00	43.50	42.50	42.00	43.75	43.25	43.14	53.75	49.50	51.63	----------
Units per 100 pounds empty body weight	6.40	7.21	7.02	5.32	9.25	6.60	4.64	6.63	4.35	4.21	4.28	----------
Ninth thoracic vertebra:												
Actual measurement----------centimeters	44.25	43.25	42.00	42.50	43.25	43.75	43.00	43.14	52.50	51.00	51.75	----------
Units per 100 pounds empty body weight	6.44	7.25	6.78	5.32	9.53	6.60	4.61	6.65	4.24	4.34	4.29	----------
Tenth thoracic vertebra:												
Actual measurement----------centimeters	41.25	42.00	41.00	40.25	42.75	43.50	39.50	41.46	48.75	48.50	48.63	----------
Units per 100 pounds empty body weight	6.00	7.04	6.62	5.04	9.42	6.56	4.23	6.42	3.94	4.12	4.03	----------
Eleventh thoracic vertebra:												
Actual measurement----------centimeters	38.00	38.00	37.00	35.75	41.00	39.50	36.25	37.93	44.75	43.50	44.13	----------
Units per 100 pounds empty body weight	5.53	6.37	5.97	4.48	9.03	5.96	3.89	5.89	3.62	3.70	3.66	----------
Twelfth thoracic vertebra:												
Actual measurement----------centimeters	31.50	33.75	30.75	28.50	37.25	34.00	28.50	32.04	40.75	38.00	39.38	----------
Units per 100 pounds empty body weight	4.58	5.66	4.96	3.57	8.21	5.13	3.05	5.02	3.29	3.23	3.26	----------
Thirteenth thoracic vertebra:												
Actual measurement----------centimeters	21.25	25.25	21.25	12.50	33.50	25.50	11.75	21.57	21.00	18.00	19.50	----------
Units per 100 pounds empty body weight	3.09	4.23	3.43	1.57	7.38	3.84	1.26	3.54	1.70	1.53	1.62	----------

Maximum length thoracic cavity:												
Actual measurement—centimeters	68.75	70.50	67.00	71.00	59.75	69.75	69.50	68.04	81.75	81.25	81.50	
Units per 100 pounds empty body weight	10.00	11.82	10.81	8.89	13.16	10.52	7.45	10.38	6.61	6.91	6.76	
Width thoracic cavity:												
At first rib:												
Actual measurement—centimeters	8.20	8.80	8.40	8.30	8.10	8.40	8.70	8.41	10.60	9.60	10.10	
Units per 100 pounds empty body weight	1.19	1.48	1.36	1.04	1.78	1.27	0.93	1.29	0.86	0.82	0.84	
At second rib:												
Actual measurement—centimeters	10.30	10.60	10.90	10.60	10.40	10.70	11.00	10.64	13.20	11.80	12.50	
Units per 100 pounds empty body weight	1.50	1.78	1.76	1.33	2.29	1.61	1.18	1.64	1.07	1.00	1.04	
At third rib:												
Actual measurement—centimeters	13.10	13.40	14.40	14.20	14.20	14.10	14.70	14.01	17.50	15.70	16.60	
Units per 100 pounds empty body weight	1.91	2.25	2.32	1.78	3.13	2.13	1.58	2.16	1.41	1.34	1.38	
At fourth rib:												
Actual measurement—centimeters	17.30	17.10	18.70	18.40	18.30	17.70	19.30	18.11	23.00	20.50	21.75	
Units per 100 pounds empty body weight	2.52	2.87	3.02	2.30	4.03	2.67	2.07	2.78	1.86	1.74	1.80	
At fifth rib:												
Actual measurement—centimeters	21.30	21.00	22.50	23.00	21.90	21.30	24.10	22.16	28.70	25.40	27.05	
Units per 100 pounds empty body weight	3.10	3.52	3.63	2.88	4.82	3.21	2.58	3.39	2.32	2.16	2.24	
At sixth rib:												
Actual measurement—centimeters	25.10	25.10	26.10	27.90	25.70	24.80	28.70	26.20	34.60	30.80	32.70	
Units per 100 pounds empty body weight	3.65	4.21	4.21	3.49	5.66	3.74	3.08	4.01	2.80	2.62	2.71	
At seventh rib:												
Actual measurement—centimeters	30.40	29.00	29.70	35.70	29.50	28.70	35.40	30.91	41.00	36.20	38.60	
Units per 100 pounds empty body weight	4.42	4.86	4.79	4.22	6.50	4.33	3.79	4.70	3.31	3.08	3.20	
At eighth rib:												
Actual measurement—centimeters	35.30	32.80	32.80	39.60	32.90	32.70	40.20	35.19	46.30	41.70	44.00	
Units per 100 pounds empty body weight	5.13	5.50	5.29	4.96	7.25	4.93	4.31	5.34	3.74	3.55	3.65	
At ninth rib:												
Actual measurement—centimeters	36.70	35.20	34.90	42.50	33.80	36.00	43.30	37.49	49.10	45.10	47.10	
Units per 100 pounds empty body weight	5.34	5.90	5.63	5.32	7.45	5.43	4.64	5.67	3.97	3.84	3.91	
Diameter of trachea outside fifth ring:												
Actual measurement—centimeters		4.52	4.09		4.25			4.29	5.17	4.56	4.87	
Units per 100 pounds empty body weight		0.76	0.66		0.94			0.79	0.42	0.39	0.41	
Diameter of trachea inside fifth ring:												
Actual measurement—centimeters		3.47	3.12		3.51			3.37	4.28	3.78	4.03	
Units per 100 pounds empty body weight		0.58	0.50		0.77			0.62	0.35	0.32	0.34	
Area of trachea inside fifth ring:												
Actual measurement—square centimeters		9.20	10.00		9.00			9.40	12.90	10.73	11.82	
Units per 100 pounds empty body weight		1.54	1.61		1.98			1.71	1.04	0.91	0.98	
Diameter of trachea outside fourth ring:												
Actual measurement—centimeters	3.48	4.52	4.11		4.27			4.10	5.21	4.63	4.92	
Units per 100 pounds empty body weight	0.51	0.76	0.66		0.94			0.72	0.42	0.39	0.41	
Diameter of trachea inside fourth ring:												
Actual measurement—centimeters	2.64	3.50	3.19		3.61			3.24	4.28	3.80	4.04	
Units per 100 pounds empty body weight	0.38	0.59	0.51		0.80			0.57	0.35	0.32	0.34	
Area of trachea inside fourth ring:												
Actual measurement—square centimeters	7.30	9.83			9.10			8.74	13.17	11.60	12.39	
Units per 100 pounds empty body weight	1.06	1.65			2.00			1.57	1.06	0.99	1.03	
Weight of thymus:												
Actual weight—grams									127.0	261.5	194.25	
Units per 100 pounds empty body weight									10.27	22.24	16.26	

TABLE 9.—*Comparative anatomy of the Herefords, Holsteins, and Aberdeen Angus*

Item	Actual units			Units per 100 pounds of empty body weight		
	Relation of Holsteins to Herefords	Relation of Aberdeen Angus to Herefords	Relation of Aberdeen Angus to Holsteins	Relation of Holsteins to Herefords	Relation of Aberdeen Angus to Herefords	Relation of Aberdeen Angus to Holsteins
	Percent	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
Live weight	+68.58	+93.77	+14.94	−5.62	−11.35	−6.07
Empty body weight	+77.69	+117.44	+22.37			
Weight of blood	+99.28			+17.74		
Weight of thyroid	+160.50	+277.30	+44.84	+36.93	+61.41	+17.88
Weight of brain	+34.57			−26.92		
Weight of pituitary body	+69.86			−6.06		
Weight of udder (empty)	+160.16	+336.08	+67.62	+50.69	+106.94	+37.33
Weight of hide	+26.20	+29.19	+2.37	−31.38	−42.67	−16.44
Weight of genital organs	−89.10	−90.35	−11.41	−92.49	−94.64	−28.57
Weight of ovaries	+112.16	+184.53	+34.11	+18.54	+30.90	+10.43
Weight of liver	+82.90	+75.18	−4.22	+1.59	−20.63	−21.87
Weight of spleen	+50.74	+49.70	−.69	−15.78	−31.26	−18.38
Weight of pancreas	+162.39	+78.45	−31.99	+45.19	−19.22	−44.37
Length of small intestine	+39.54	+23.93	−11.19	−23.70	−44.54	−27.31
Length of large intestine	+48.37	+38.21	−6.85	−18.18	−37.69	−23.85
Total length of intestines	+41.39	+26.93	−10.23	−22.55	−43.11	−26.55
Weight of small intestine	+11.12	−15.86	−24.35	−39.49	−62.42	−37.89
Weight of large intestine	−9.20	−21.64	−13.70	−49.66	−64.63	−29.73
Total weight of intestines	+1.24	−18.69	−19.68	−44.59	−63.61	−34.32
Weight of paunch	+70.50	+22.84	−27.95	−5.49	−44.51	−41.28
Weight of reticulum	+56.00	+42.50	−8.65	−13.33	−36.67	−26.92
Weight of omasum	+55.59	+19.89	−22.94	−14.55	−45.45	−36.17
Weight of abomasum	+3.28	+19.95	+16.14	−45.16	−48.39	−5.88
Total weight of stomachs	+54.51	+23.04	−20.37	−15.36	−44.79	−34.77
Total weight of abdominal fat	+410.43	+599.44	+37.03	+207.51	+241.04	+10.90
Weight of pluck	+121.03	+106.39	−6.62	+22.28	−6.74	−23.73
Weight of right lung	+79.58	+10.92	−38.24	−.00	−51.16	−51.16
Weight of left lung	+72.81	+24.42	−28.00	−6.06	−45.45	−41.94
Total weight of lungs	+76.65	+16.77	−33.90	−2.63	−47.37	−45.95
Weight of heart, auricles attached	+103.57	+70.63	−16.18	+13.16	−23.68	−32.56
Circumference of heart (base)	+27.78	+26.05	−1.35	−29.32	−43.16	−19.58
Circumference of heart (apex 1)	+27.95	+17.46	−8.20	−29.47	−47.02	−24.89
Circumference of heart (apex 2)	+29.85	+16.60	−10.20	−28.55	−47.54	−26.57
Weight of heart, auricles removed	+103.06	+70.31	−16.13	+14.71	−23.53	−33.33
Total weight of thoracic fat	+288.89	+322.71	+8.70	+134.48	+103.45	−13.24
Weight of kidneys	+79.22	+160.00	+45.08	−1.73	+16.70	+18.75
Weight of adrenals	+63.87	+211.48	+90.08	−12.59	+36.01	+55.60
Weight of dressed carcass	+84.48			+3.32		
Depth of thoracic cavity:						
At first thoracic vertebra	+25.59			−31.28		
At second thoracic vertebra	+13.58			−38.55		
At third thoracic vertebra	+21.69			−34.13		
At fourth thoracic vertebra	+22.75			−33.47		
At fifth thoracic vertebra	+19.99			−35.00		
At sixth thoracic vertebra	+19.78			−35.44		
At seventh thoracic vertebra	+19.19			−35.65		
At eighth thoracic vertebra	+19.68			−35.44		
At ninth thoracic vertebra	+19.96			−35.49		
At tenth thoracic vertebra	+17.29			−37.23		
At eleventh thoracic vertebra	+16.35			−37.86		
At twelfth thoracic vertebra	+22.91			−35.06		
At thirteenth thoracic vertebra	−9.60			−54.24		
Maximum length of thoracic cavity	+19.78			−34.87		
Width of thoracic cavity:						
At first rib	+20.10			−34.88		
At second rib	+17.48			−36.59		
At third rib	+18.49			−36.11		
At fourth rib	+20.10			−35.25		
At fifth rib	+22.07			−33.92		
At sixth rib	+24.81			−32.42		
At seventh rib	+24.88			−31.91		
At eighth rib	+25.04			−31.65		
At ninth rib	+25.63			−31.04		
Diameter of trachea outside fifth ring	+13.52			−48.10		
Diameter of trachea inside fifth ring	+19.58			−45.16		
Area of trachea inside fifth ring	+25.74			−42.69		
Diameter of trachea outside fourth ring	+20.00			−43.06		
Diameter of trachea inside fourth ring	+24.69			−40.35		
Area of trachea inside fourth ring	+41.76			−34.39		

PERCENTAGE DIFFERENCE IN ACTUAL UNITS OF WEIGHT OR MEASUREMENT

The percentages in table 9 indicate that on the basis of actual units, the Holsteins exceeded the Herefords by more than 100 percent in nine items, the greatest differences being, in order of magnitude, weights of total abdominal fat, total thoracic fat, pancreas, thyroid, udder, pluck,[4] ovaries, and the two weights of the heart. The only items differing by less than 10 percent are weights of total intestine, abomasum, and large intestine, and one of the depths of the thoracic cavity.

The Aberdeen Angus exceeded the Herefords by more than 100 percent for nine items. Three of these (the weights of total abdominal fat, total thoracic fat, and the udder) were above 300 percent. In this connection an extremely fatty condition of the udder is noted, indicating that the greatest differences are in the parts made up chiefly of body fat. Two others (the weights of thyroid and of adrenals) were above 200 percent, suggesting a marked difference in some of the endocrine glands. The other four in the order of magnitude are weights of ovaries, kidneys, empty body, and pluck. Deposition of fat could have been at least partly responsible for the high percentage of each of these nine items, except for the weight of ovaries. None of the items in this comparison had a percentage below 10 percent. Of the five smallest percentages, which ranged from 10.92 for weight of right lung to 17.46 for circumference of heart, four were for weights or measurements of organs of circulation and respiration.

When the Aberdeen Angus is compared with the Holsteins the differences are much less marked. In no case is the difference as much as 100 percent. The four highest percentages, all of which show excesses for the Aberdeen Angus, range from 90.08 to 44.84 and include in the order of their magnitude the weights of adrenals, udder, kidneys, and thyroid. On the other hand nine items differ by less than 10 percent and only two of them are greater for the Aberdeen Angus than for the Holsteins.

In comparing the Aberdeen Angus cow with cows of the other breeds—especially the Herefords—the extreme differences in condition of flesh should be kept in mind. Undoubtedly these differences in flesh are responsible for some of the differences found in both the ante-mortem and post-mortem data.

PERCENTAGE DIFFERENCES IN UNITS OF WEIGHT OR MEASUREMENT PER 100 POUNDS OF EMPTY BODY WEIGHT

When the comparison of breeds was made on the basis of units per 100 pounds of empty body weight, the Holsteins differed from the Herefords by as much as 100 percent only for weight of abdominal and thoracic fats. Both were greater for the Holsteins than for the Herefords. For nine items the difference was less than 10 percent. Of these, six were greater for the Herefords. Values that were lower for Holsteins than for Herefords on the basis of actual units became even lower on this basis, and those that were positive on the actual unit basis showed either lower positive or negative values when compared on the basis of empty body weight. The effect of degree of fleshing on the empty body weight may have been to some extent responsible for these differences between the two bases of comparison.

[4] In this study the word "pluck" refers to the unseparated contents of the thoracic cavity as removed in slaughtering.

A comparison of the Aberdeen Angus with the Herefords shows only three items (weight of abdominal and thoracic fats and the udder) differing by more than 100 percent, all of which are lower for the Herefords than for the Aberdeen Angus. Only one item (the weight of pluck) differs by less than 10 percent. As in comparing the Holsteins with the Herefords, all items that were positive on the basis of actual units became either lower positive or negative, and those that were negative became negative to an even greater degree. Deposition of fat, or fleshing, seems to have been chiefly responsible for the differences shown by the two methods of comparison.

A comparison of the Aberdeen Angus with the Holsteins shows that no item of measurement differs by as much as 60 percent, only two differ by more than 50 percent, and only two differ by less than 10 percent. The relation between the values based on actual units and those based on units per 100 pounds of empty body weight in this breed comparison is similar to that shown in the other breed comparisons and the same factors are probably responsible.

PERCENTAGE DIFFERENCES IN GROUPS OF ITEMS COMBINED ON THE BASIS OF ANATOMY AND FUNCTION

To obtain an idea of the extent to which differences in anatomy are associated with the functions of the organs and body parts, the items in table 9 were divided into seven groups as follows: (1) The body-size group (skeletal and muscular), which includes the live weight, empty body weight, weight of hide and dressed carcass, and the carcass measurements; (2) the nervous-system group, which includes the weight of brain; (3) the circulation and respiration group, which includes weight of blood, pluck, lungs, and heart, and the measurements of the heart and trachea; (4) the digestion group, which includes the weight of liver, pancreas, intestines and stomachs, and the intestine measurements; (5) the urogenital group, which includes the weight of udder, genital organs, ovaries, and kidneys; (6) the endocrine gland group, which includes the weight of the thyroid, pituitary body, spleen, and adrenals; and (7) the visceral fats group, which includes the abdominal and thoracic fats.

The average of the differences expressed in percentage for the items in each group and for each breed comparison, was determined first with the plus and minus signs disregarded, and second with the plus and minus signs considered. The averages are given in table 10.

The organs and parts of the Holsteins and the Aberdeen Angus were actually of greater magnitude than those of the Herefords for all groups, whereas those of the Aberdeen Angus were smaller than those of the Holsteins in the circulation and respiration and the digestion groups. However, on the basis of units per 100 pounds of empty body weight, which measures the proportion of the different organs and parts to the total animal structure, the values were greater for the Herefords than for the Holsteins in all except the endocrine gland and visceral fats groups; greater for the Herefords than for the Aberdeen Angus in all except the urogenital, the endocrine gland, and the visceral fats groups; and greater for the Aberdeen Angus than for the Holsteins only in the urogenital and endocrine gland groups. The effect of greater fleshing is clearly shown by these results, for as the total animal structure becomes greater as a result of heavier fleshing, the organs and parts become proportionately smaller. The only group

TABLE 10.—*Degree of difference between breeds for post-mortem items grouped on basis of anatomy and function*

Item	Actual units						Units per 100 pounds of empty body weight					
	Holsteins differ from Herefords		Aberdeen Angus differs from Herefords		Aberdeen Angus differs from Holsteins		Holsteins differ from Herefords		Aberdeen Angus differs from Herefords		Aberdeen Angus differs from Holsteins	
	Items	Average	Items	Average	Items	Average	Items	Average	Items	Average	Items	Average
Plus and minus signs disregarded in determining averages:	*Number*	*Percent*	*Number*	*Percent*	*Number*	*Percent*	*Number*	*Percent*	*Number*	*Percent*	*Number*	*Percent*
Body size	27	26.80	3	80.13	3	13.23	26	32.99	2	27.01	2	11.26
Nervous system	1	34.57					1	26.92				
Circulation and respiration	16	55.43	9	39.95	9	17.65	16	26.10	9	37.29	9	33.30
Digestion	13	48.93	13	32.85	13	16.79	13	26.06	13	44.28	13	30.07
Urogenital	4	110.16	4	192.74	4	39.56	4	40.86	4	62.30	4	23.77
Endocrine gland	4	86.24	3	179.49	3	45.20	4	17.84	3	42.89	3	30.62
Visceral fats	2	349.66	2	461.08	2	22.87	2	171.00	2	172.25	2	12.07
All groups combined	67	56.20	34	95.82	34	22.24	66	33.61	33	51.14	33	28.01
Plus and minus signs considered in determining averages:												
Body size	27	+26.09	3	+80.13	3	+13.23	26	−32.86	2	−27.01	2	−11.26
Nervous system	1	+34.57					1	−26.92				
Circulation and respiration	16	+55.43	9	+39.95	9	−17.65	16	−17.62	9	−37.29	9	−33.30
Digestion	13	+47.51	13	+24.21	13	−14.31	13	−18.87	13	−44.28	13	−30.07
Urogenital	4	+65.61	4	+147.57	4	+33.85	4	−6.25	4	+14.98	4	+9.49
Endocrine gland	4	+86.24	3	+179.49	3	+44.74	5	+.63	3	+22.05	3	+18.37
Visceral fats	2	+349.66	2	+461.08	2	+22.87	2	+171.00	2	+172.25	2	−1.17
All groups combined	67	+52.98	34	+87.20	34	+.30	66	−16.45	33	−14.99	33	−18.86

of items which consistently overcame this factor and became greater for Holsteins than for Herefords, and greater for Aberdeen Angus than for either Herefords or Holsteins was the one which included the endocrine glands.

UDDER CHARACTERISTICS AS JUDGED BY ANTE-MORTEM EXAMINATION

Ante-mortem examinations of the udders of the cows used in this study were made in accordance with the general plan adopted by the Bureau of Dairy Industry for studying the udders of the animals in the breeding herd at Beltsville, Md. As explained in a previous publication (*10*), evaluation of the different items that cannot be definitely measured is accomplished by assigning grades ranging from 1 to 9. In each case a grade of 9 indicates the maximum of the characteristic under consideration but does not necessarily signify excellence. In some cases a grade is followed by a plus or a minus sign indicating that the item rates slightly above or below the grade given.

The terms used to describe the udder characteristics compared in this study are defined as follows:

Looseness of udder—the extent to which its covering can be stretched.

Compressibility of udder—the degree to which the udder as a whole responds to pressure.

Abundance of swelling—abundance of congestion resulting from injury, infection, or incidental to parturition.

Abundance of "make-up"—abundance of the substance often present in the udder before parturition and remaining in varying degrees and for varying periods thereafter.

Abundance of gland tissue—quantity of gland tissue in the udder, considering breed and size of animal, stage of lactation, and other factors.

Length of halves—the distance between the anterior and posterior attachments of the gland tissue.

Width of halves—the width of each half above the teats.

Depth of halves—the distance from the abdominal wall to the lowest extremity of the gland tissue.

Nearness to surface—freedom both of deposits of fat around the gland tissue, and of congestion incidental to parturition.

Distinctness of outline—ease with which the outlines and boundaries of the gland tissue can be determined.

Compressibility of gland tissue—the degree to which it responds to pressure.

Mellowness of gland tissue—the degree to which it responds to kneading.

Abundance of fiber—the extent to which the gland tissue is corrugated on the surface, or composed of ropelike strands of tissue.

Coarseness of fiber—the size of the corrugations or strands.

Harshness of fiber—the resistance or harshness of the corrugations or strands.

Stringiness of fiber—the looseness with which the ropelike strands of fiber are held together. In a stringy udder the tissue is compressible and sometimes mellow; in one which is fibrous but not stringy the tissue usually is firm, full, and appears corrugated on the surface.

Openness of cisterns—the size of the openings between the teat canals and the cisterns.

Free space—the distance above the base of the teat which can readily be compressed without marked resistance.

Udder veins—the abundance of veining on the surface of the udder.

Abdominal veins—the abundance of veining on the surface of the abdomen (milk veins).

Milk wells—the size of the openings in the abdominal wall through which the "abdominal" veins pass.

The data representing the different udder characteristics are given in table 11.

TABLE 11.—*Udder characteristics (ante-mortem) of the animals used in the experiment*

Item	Basis of evaluation	Herefords							Holsteins		Aberdeen Angus
		Group 1 (dairy management)				Group 2 (beef management)					
		H-2	H-5	H-6	H-16	H-1	H-4	H-17	No. 299	No. 827	A-300
Looseness of udder	Grade	5	6	6	4	4	4	5	9	6	8
Yieldability of udder	do	7	7	7	7	7	7	6	8	9	7
Abundance of swelling	do	1—	1—	1	1	1—	1	1	1—	1—	------
Abundance of "make-up"	do	1	1	1	1—	1	1—	4	1	1—	------
Abundance of gland tissue	do	1+	2	1	2	2	1—	3	3	2—	5
Length of halves	Inches	6.00	4.50	5.50	8.00	5.00	4.00	9.00	------	------	------
Width of halves:											
Above front teat	do	1.00	1.00	1.00	1.25	1.25	1.00	2.00	------	------	------
Above rear teat	do	1.25	1.00	1.00	1.25	1.25	1.00	2.00	------	------	------
Average	do	1.13	1.00	1.00	1.25	1.25	1.00	2.00	------	------	------
Depth of halves	Grade	2	4	2	4	2	1	3	------	------	------
Nearness to surface	do	5	7	6	5	7	4	4	6	8	4
Distinctness of outline	do	5	7	5	5	7	3	4	7	8	5
Compressibility of gland tissue.	do	5	6	4	6	5	5	4	8	8	4
Mellowness of gland tissue	do	5	6	4	6	5	5	4	8	7	4
Abundance of fiber	do	6	6	5	5	7	5	5	6	6	4
Coarseness of fiber	do	5	6	5	5	6	5	5	4	5	6
Harshness of fiber	do	6	6	5	4	6	6	5	5	5	------
Stringiness of fiber	do	4	4	3	4	4	5	4	4	3	5
Openness of cisterns:											
Left front quarter	do	1	1	1	1	5	1	2	------	3	------
Right front quarter	do	1	1	1	1	5	1	2	------	3	------
Left rear quarter	do	3	1	1	2	1	1	6	------	5	------
Right rear quarter	do	2	1	2	2	2	1	7	------	5—	------
Average	do	1.75	1.00	1.25	1.50	3.25	1.00	4.25	------	4.00	------
Free space:											
Left front quarter	Inches	3.25	3.50	3.50	4.25	3.75	3.25	3.50	4.50	4.75	3.25
Right front quarter	do	3.50	3.50	3.50	4.25	3.75	3.25	3.50	4.50	4.75	3.00
Left rear quarter	do	3.25	3.50	3.25	4.50	3.75	3.25	3.50	4.25	4.75	5.00
Right rear quarter	do	3.00	3.50	3.25	4.25	4.25	3.25	3.50	4.50	4.75	5.00
Average	do	3.25	3.50	3.38	4.31	3.88	3.25	3.50	4.44	4.75	4.06
Udder veins:											
Right	Grade	1	1	1	2	1	2	2	5	3	1—
Left	do	1	1	1	2	1	2	2	4	2	1—
Abdominal veins:											
Right	do	1	2	2	3	3	3	4	6	5	1—
Left	do	3	3	1	2	3	3	4	7	7—	1—
Milk wells:											
Right	do	1	2	1	2	2	1	2	7	3—	1
Left	do	2	3	1—	2	1	1	2	7	3	6

The estimates on the various udder characteristics, as given in table 11, were made on all the animals while they were dry and shortly before they were slaughtered. The length of time the individual animals had been dry varied greatly.

In the estimates of those udder characteristics that are associated with "quality of udder" (i. e., looseness of udder, yieldability of udder, compressibility of gland tissue, mellowness of gland tissue, stringiness of fiber, and free space), there was no great difference between the two groups of Herefords. The two Holstein cows averaged considerably higher than the Herefords for most of these items, although for some items individual Herefords were graded as high as the Holsteins. The Aberdeen Angus cow was close to the average of the Herefords for

most of these items. For looseness of udder and stringiness of fiber, however, she was given a higher rating than the Holsteins.

In those characteristics that might be termed antagonistic to quality of udder (such as abundance, coarseness, and harshness of fiber) there was no great difference on the average between the grades for the two groups of Herefords, the Holsteins, and the Aberdeen Angus.

The greatest and most consistent differences between the Holsteins and the Herefords and the Aberdeen Angus were in the abdominal veins, milk wells, and udder veins, for which the Holsteins were definitely superior. This may be to some extent indicative of the greater blood flow from the udder.

Although the grades for quality of udder are shown to be greater for the Holsteins than for the Herefords or the Aberdeen Angus, the differences do not approach the great difference in producing capacity that actually existed between the Holsteins and the Herefords, and that presumably also existed between the Holsteins and the Aberdeen Angus.

POST-MORTEM STUDIES OF THE UDDER

SIZE AND CAPACITY

The method of studying the udder after its amputation was described in considerable detail in a previous publication (*10*). Size of udder refers to its empty weight. Capacity of udder is the quantity of formalin held by the secretory system of the udder expressed in terms of equivalent weight of milk. The relation of capacity to weight of udder, expressed as a percentage, is obtained by dividing the capacity by the empty weight and multiplying the result by 100. It is a measure of the fluid-holding capacity of the secretory system per unit of udder weight. Data for size, capacity, and relation of capacity to weight, for the Herefords, the Holsteins, and the Aberdeen Angus are given in table 12.

In empty weight of udder the two groups of Herefords differed but slightly, group 2 being only 2.30 percent greater than group 1; the Holsteins averaged 160.16 percent greater than the Herefords; the Aberdeen Angus was 336.08 percent greater than the Herefords and 67.62 percent greater than the Holsteins.

In capacity of udder the Herefords in group 2 averaged 53.32 percent greater than those in group 1; the Holsteins averaged 520.33 percent greater than all the Herefords; and 359.78 percent greater than the Aberdeen Angus; the Aberdeen Angus averaged 34.92 percent greater than the Herefords.

For the values representing the relation of capacity to weight of udder, the Herefords in group 2 averaged 74.86 percent higher than those in group 1; the Holsteins average 130.12 percent higher than the Herefords; the Aberdeen Angus was 69.96 percent lower than the Herefords, and 86.95 percent lower than the Holsteins.

It has been found (*4, Rept. 1932, p. 7*) that the capacity of lactating udders is considerably greater than that of nonlactating udders. All the cows in this study were nonlactating, but the period of time since being dried off varied to some extent. It is not at all unlikely that the prolonged inactivity of the Aberdeen Angus may have resulted in a greater deposition of fat around the gland, which would reduce the value for relation of capacity to weight of udder even though the capacity itself might not have been greatly affected. It might appear

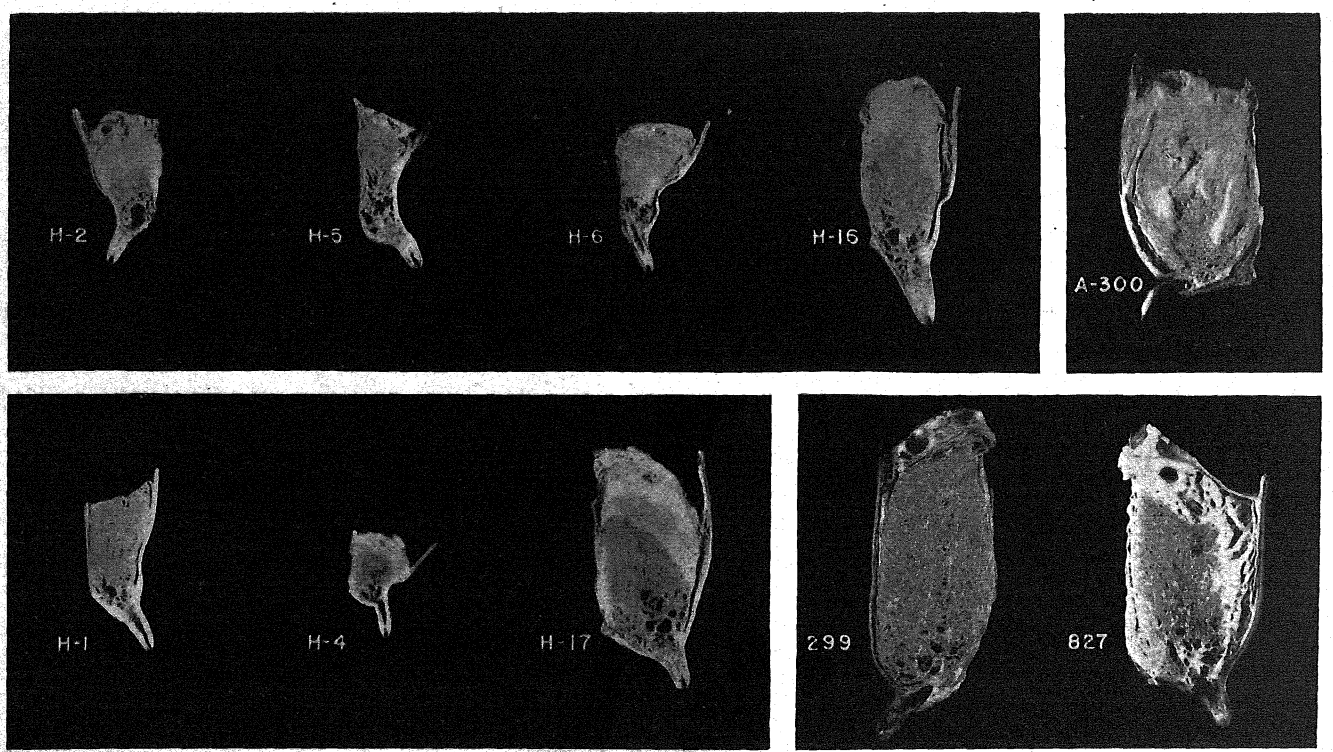
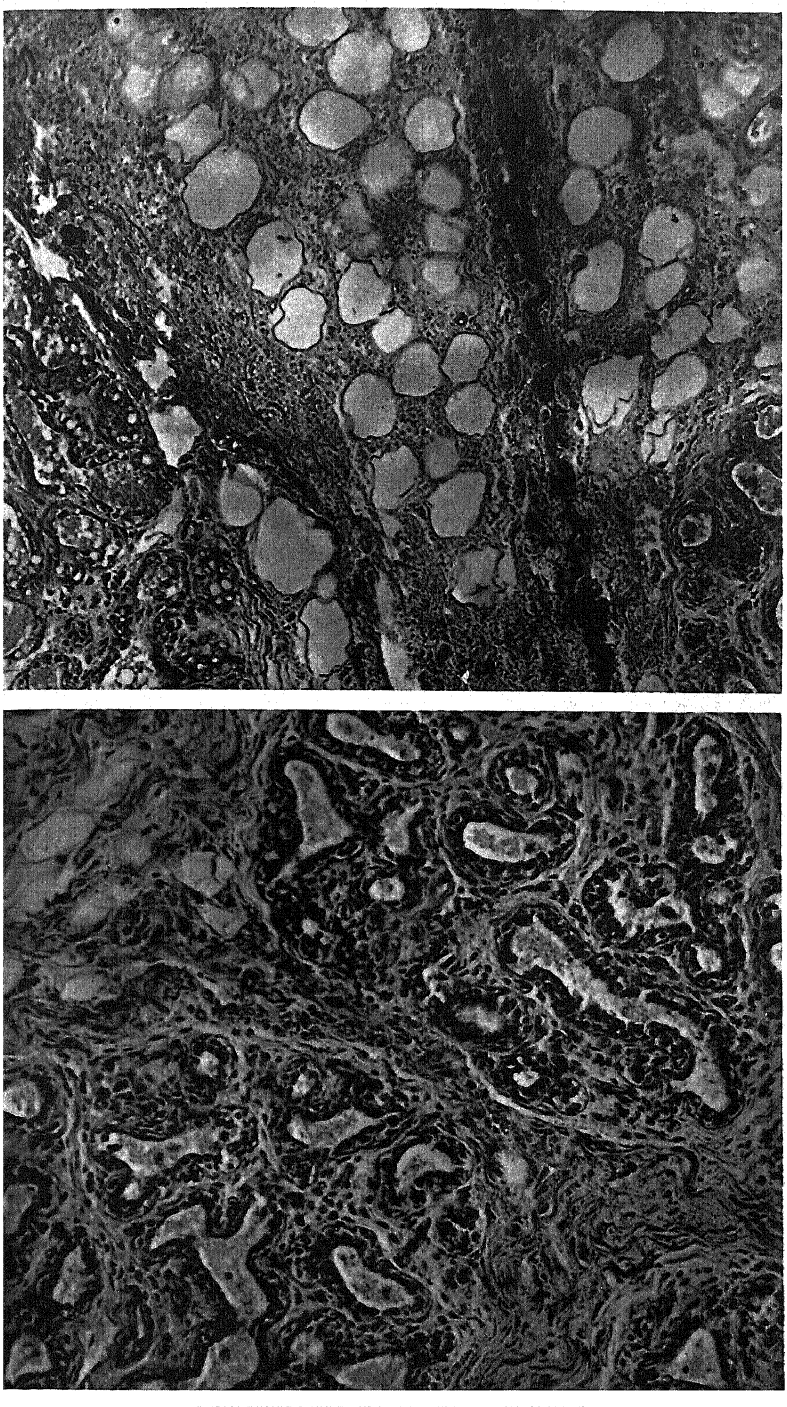


PLATE 1

Vertical transverse sections through rear quarter of each of the 10 udders studied, reduced to same scale to show comparative size. In some a large proportion of the area consists of body fat rather than glandular tissue.



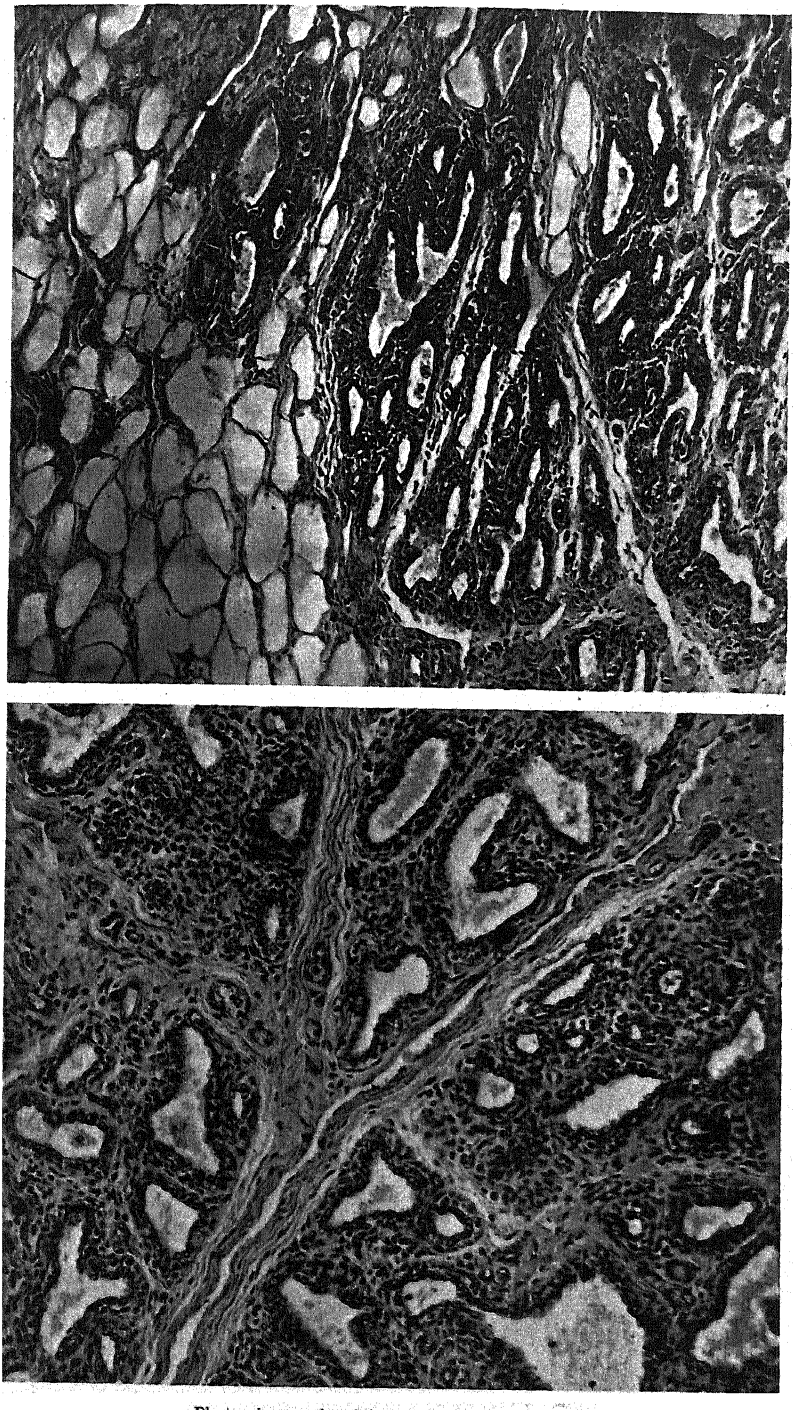
Vertical transverse section through a rear quarter of the udder of H-2.



Photomicrographs of tissue from the udder of H–2.



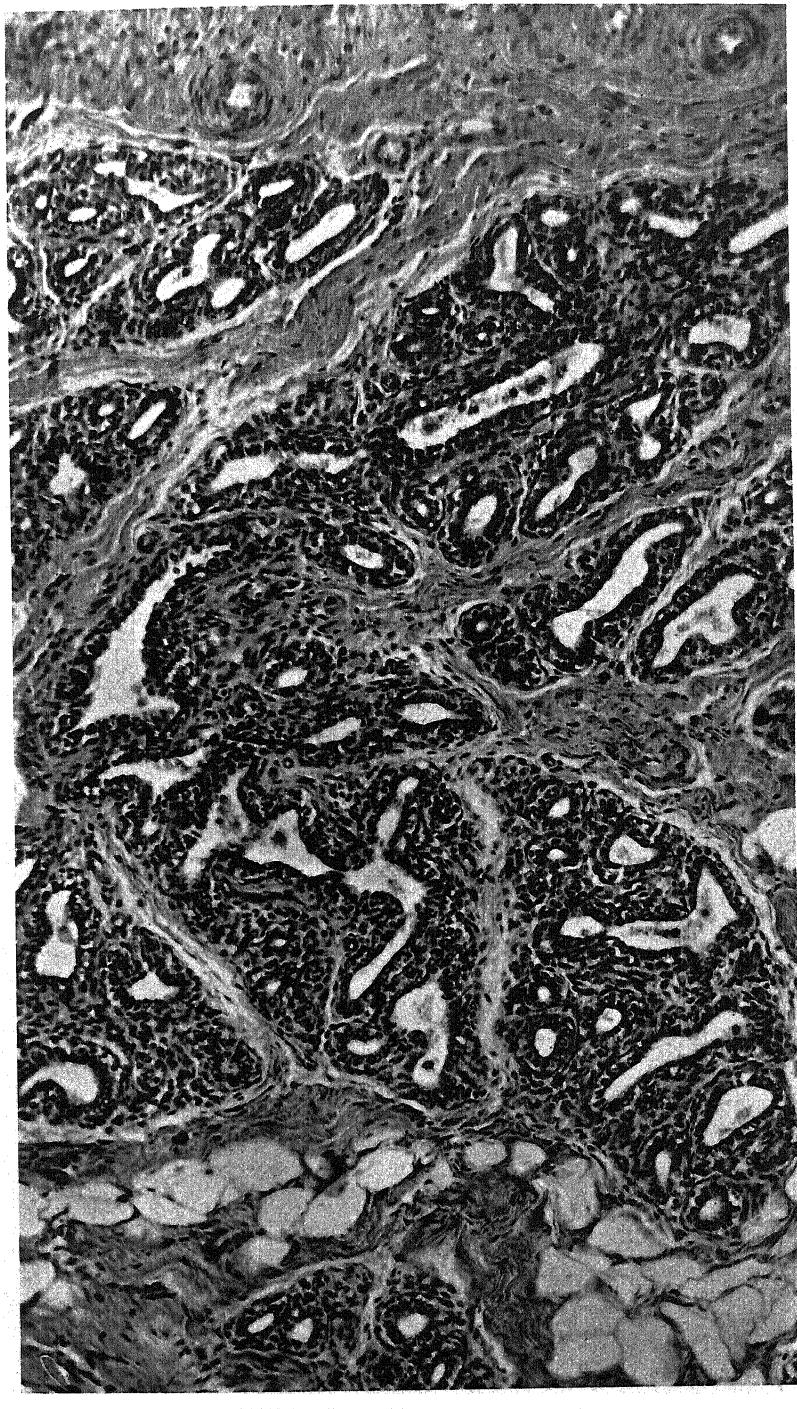
Vertical transverse section through a rear quarter of the udder of H-5.



Photomicrographs of tissue from the udder of H-5.



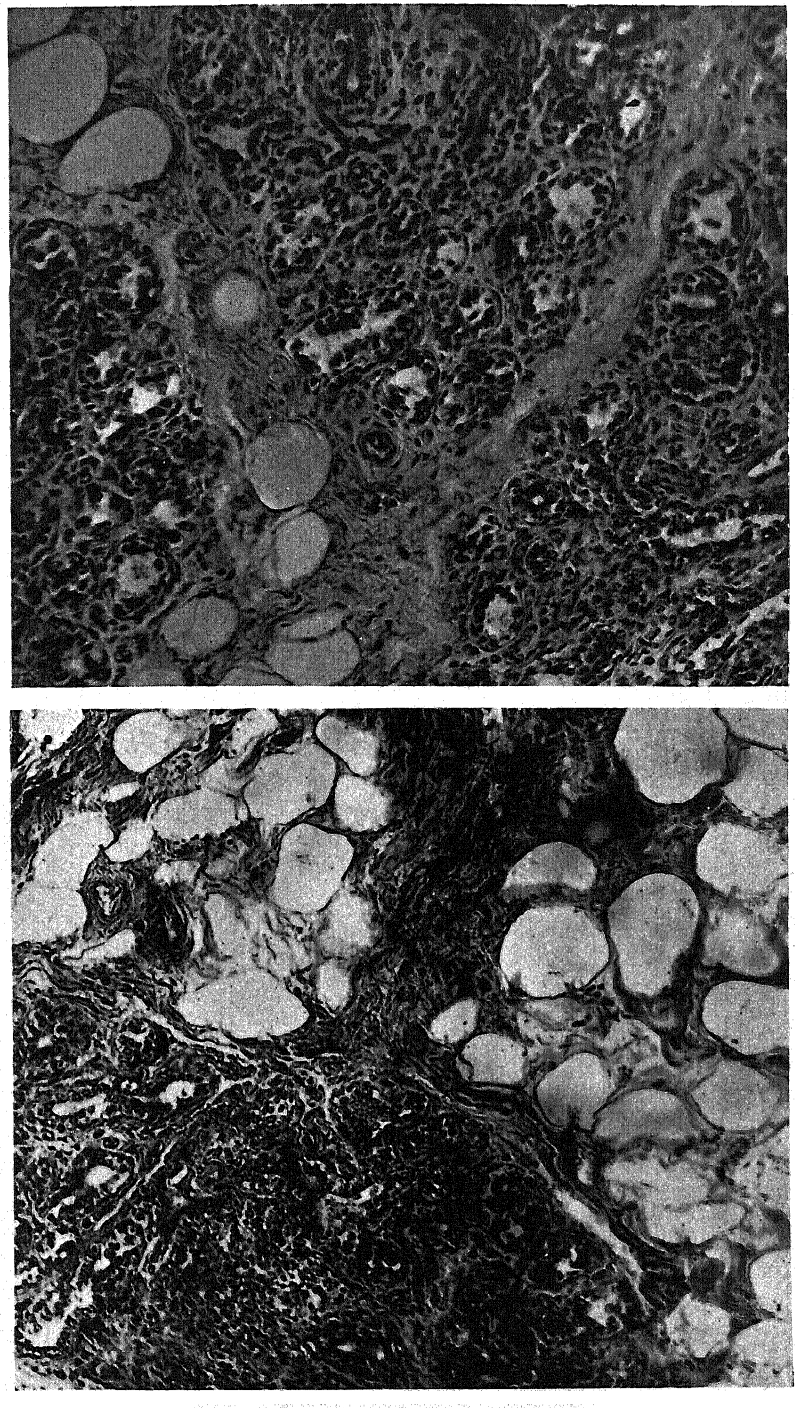
Vertical transverse section through a rear quarter of the udder of H–6.



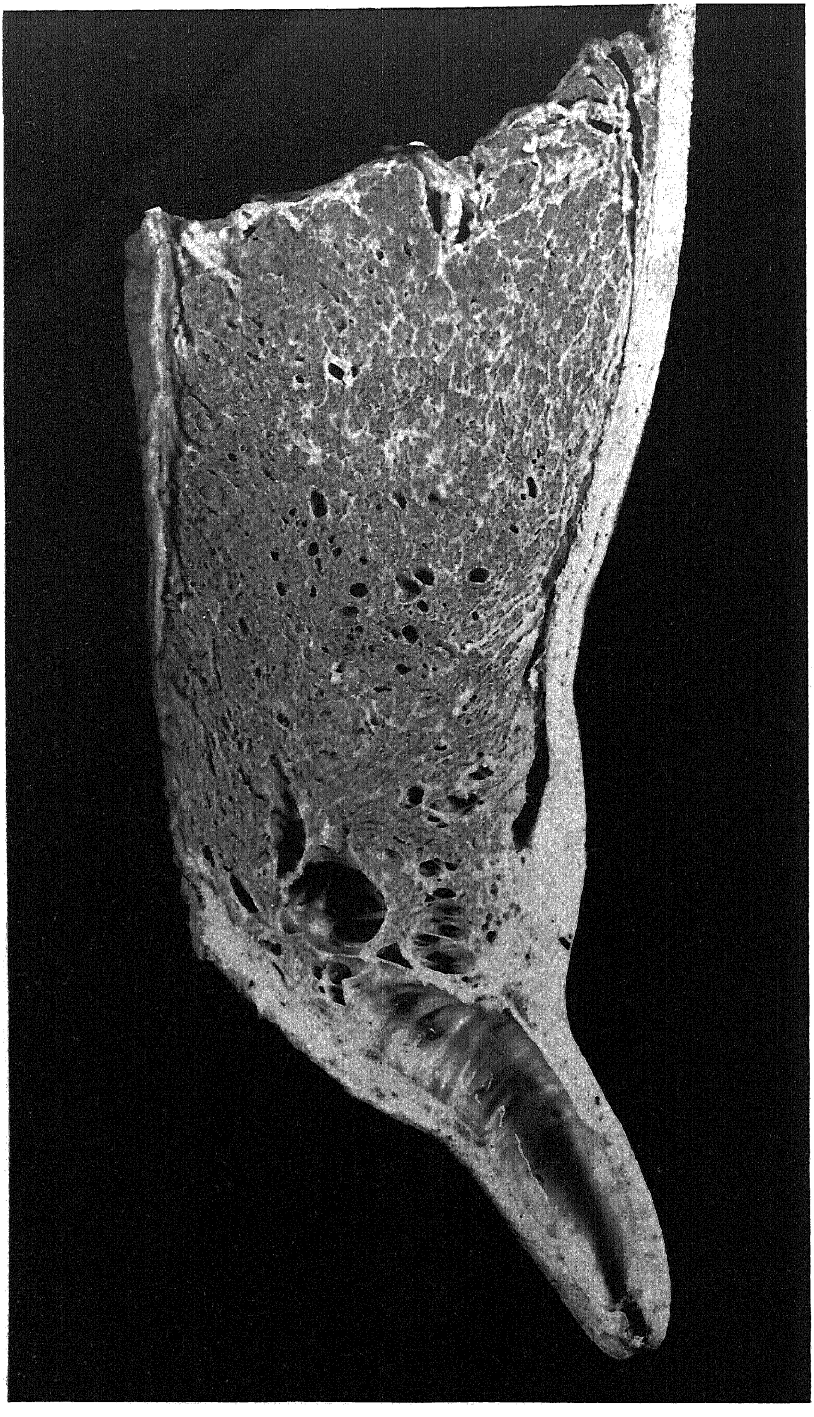
Photomicrograph of tissue from the udder of H–6.



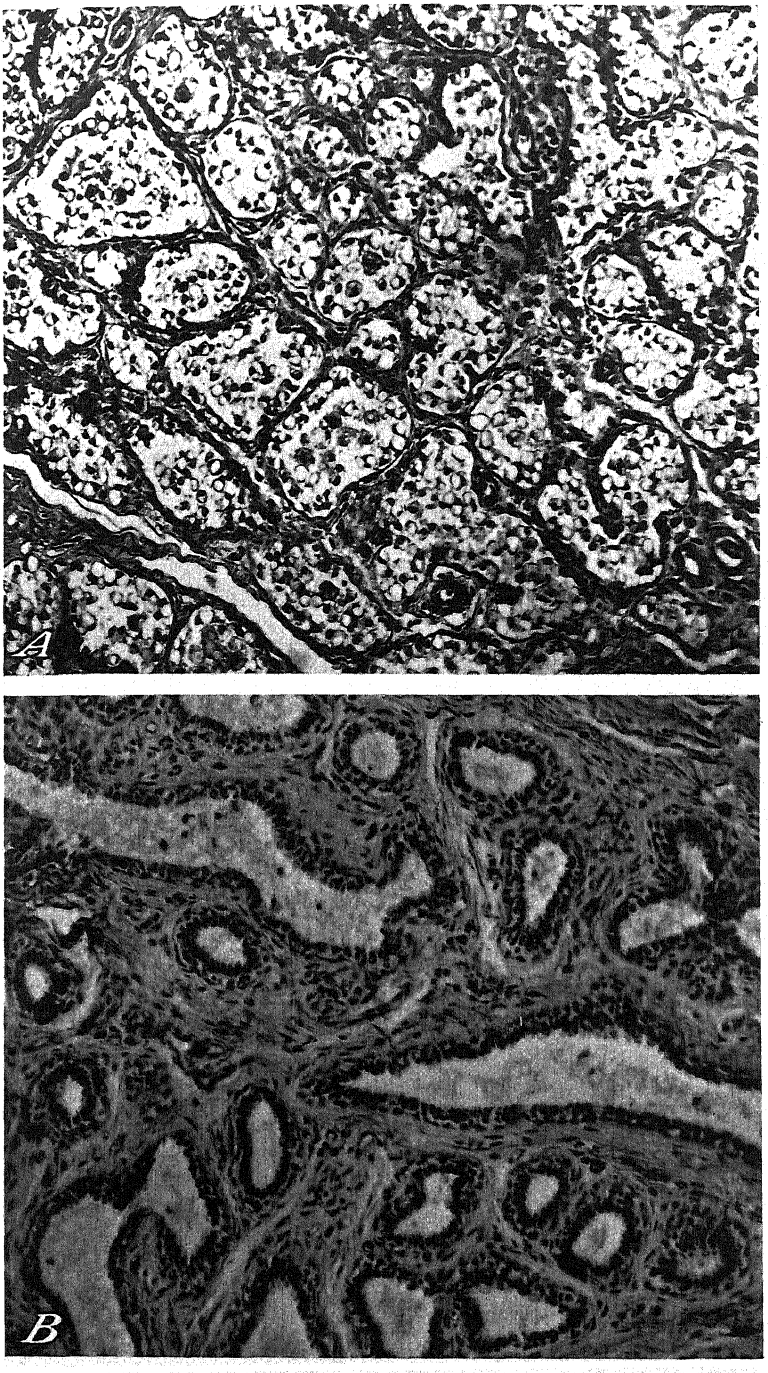
Vertical transverse section of a rear quarter of the udder of H–16.



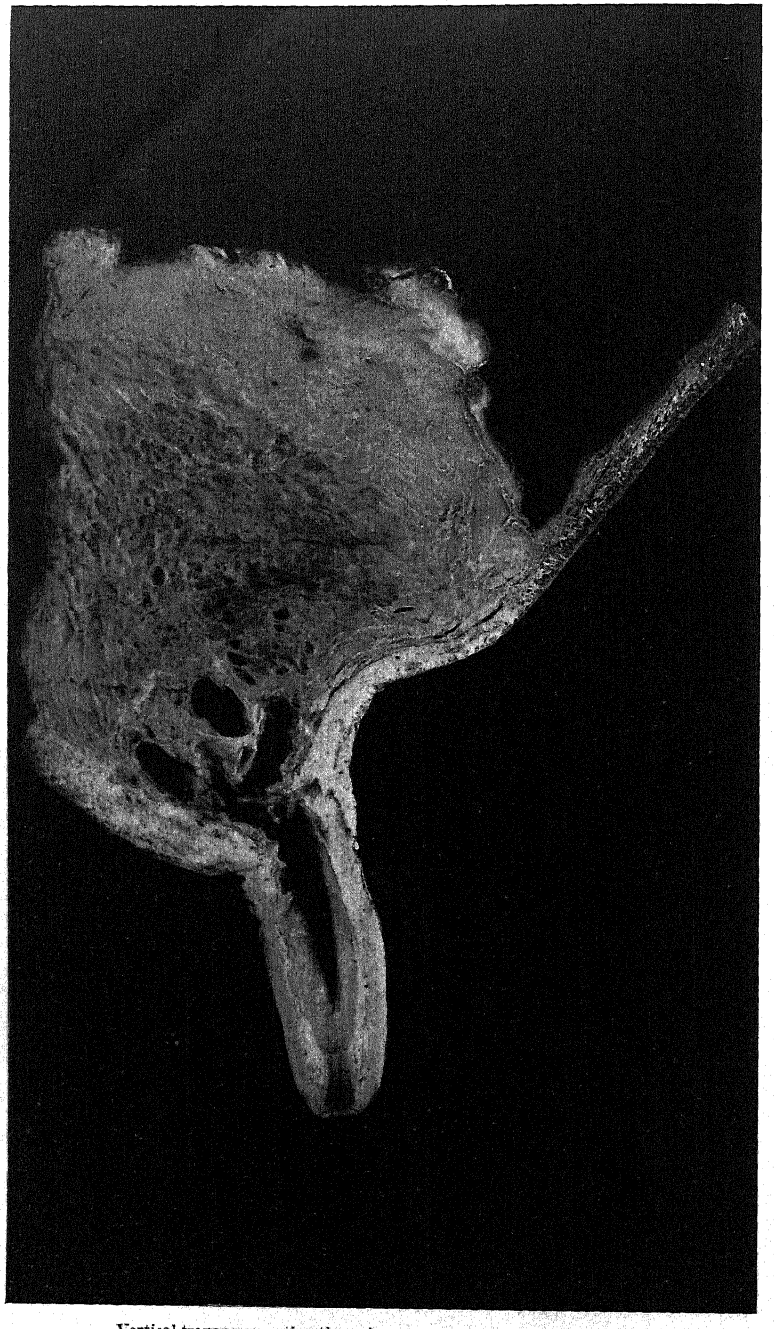
Photomicrographs of tissue from the udder of H-16.



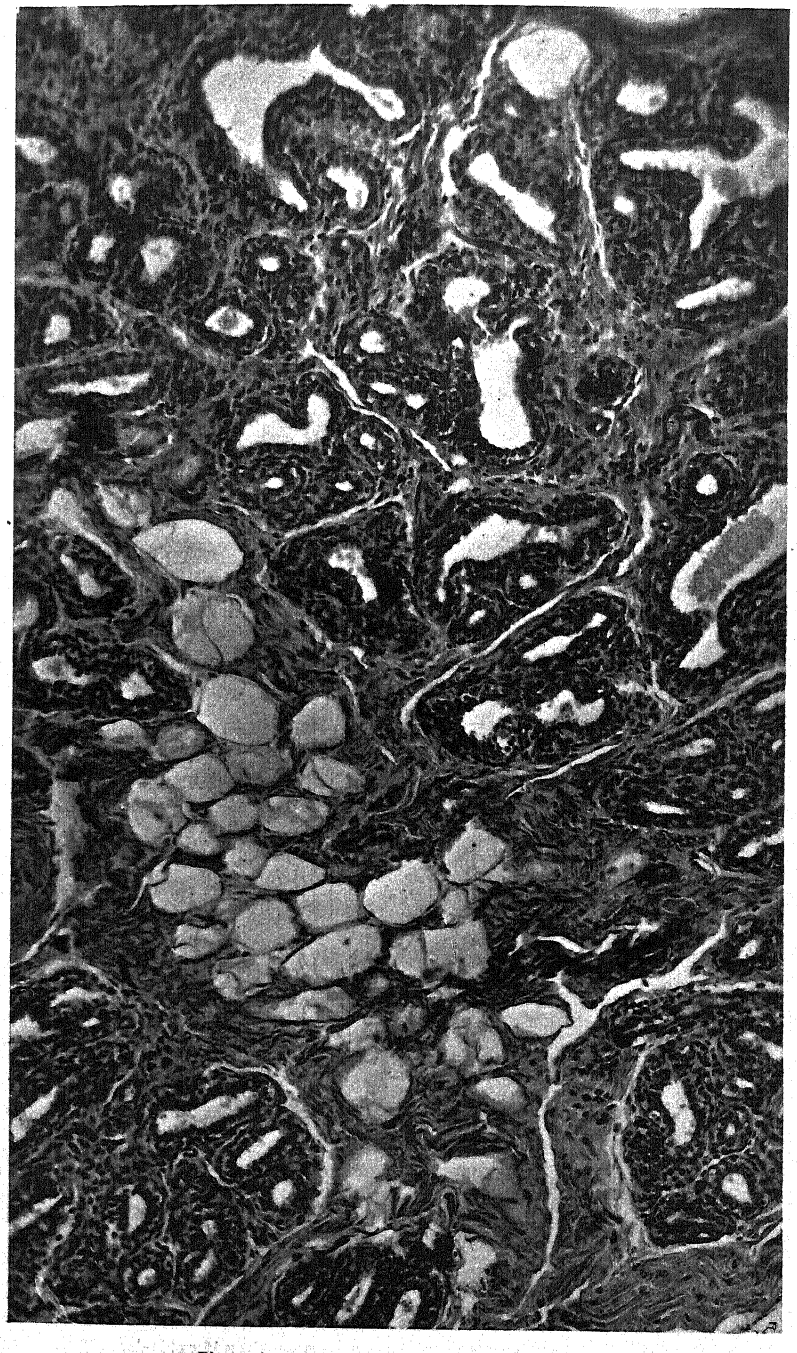
Vertical transverse section through a rear quarter of the udder of H-1.



Photomicrographs of tissue from the udder of H–1: *A*, Functioning tissue from the upper portion of the front quarter; *B*, nonfunctioning tissue from the lower portion of the rear quarter.



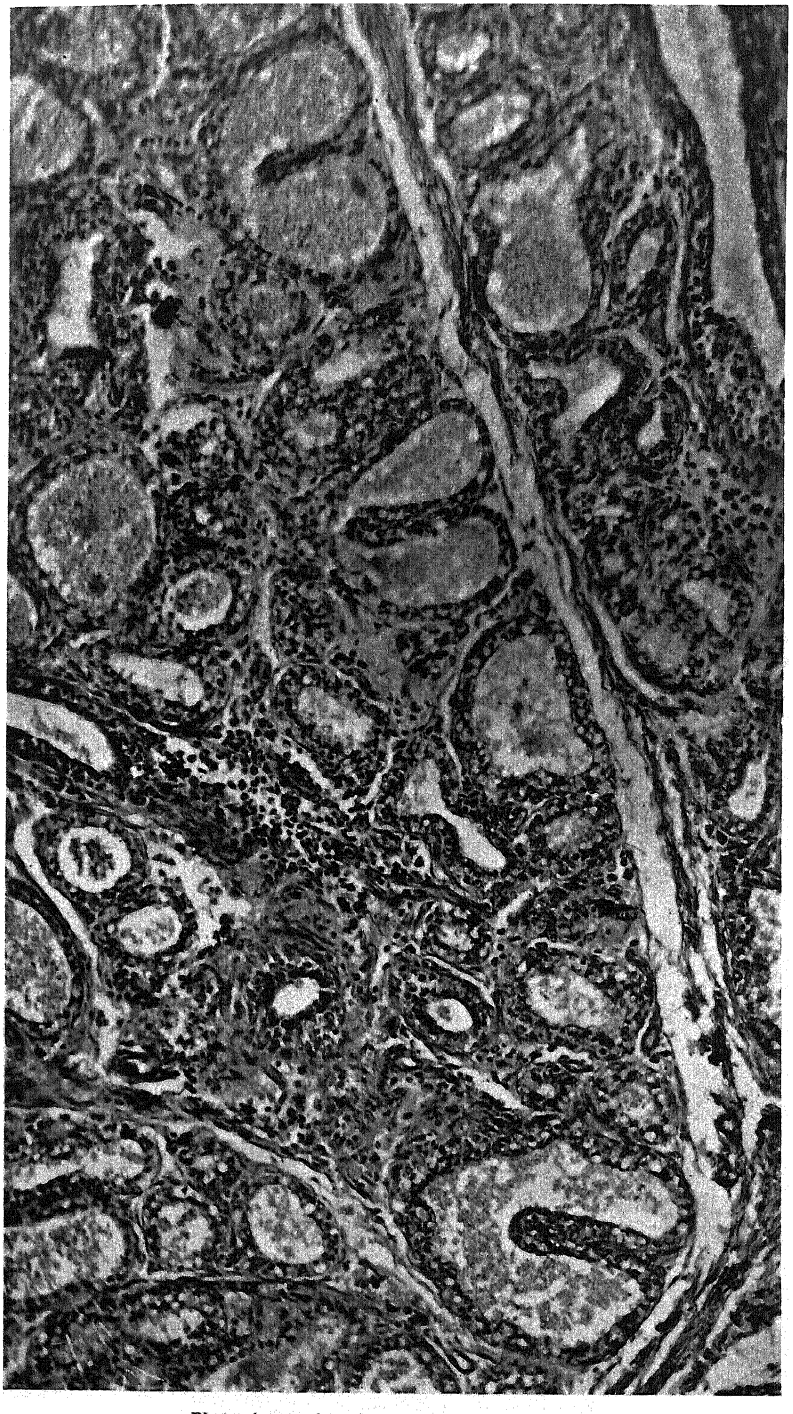
Vertical transverse section through a rear quarter of the udder of H-4.



Photomicrograph of tissue from the udder of H-4.



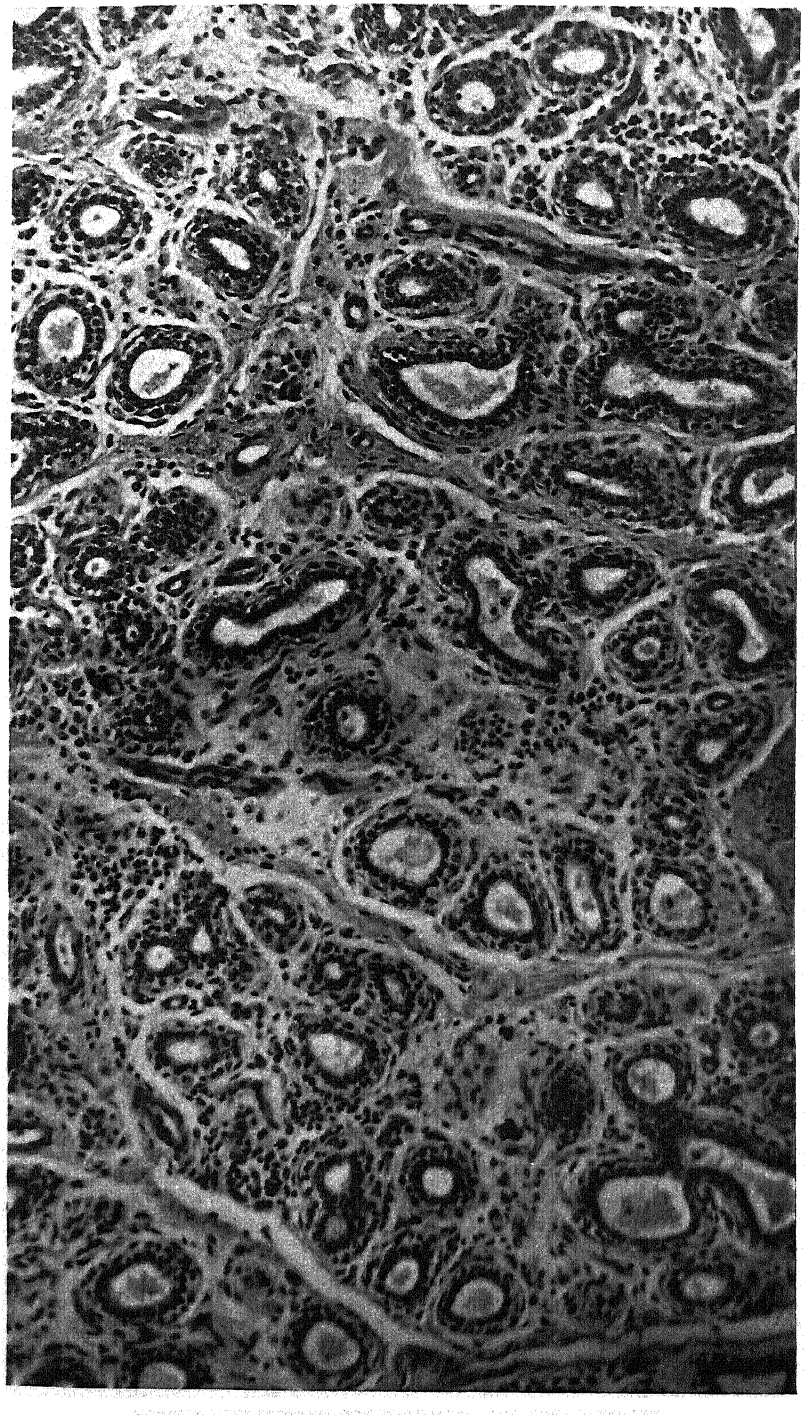
Vertical transverse section through a rear quarter of the udder of H–17.



Photomicrograph of tissue from the udder of H-17.



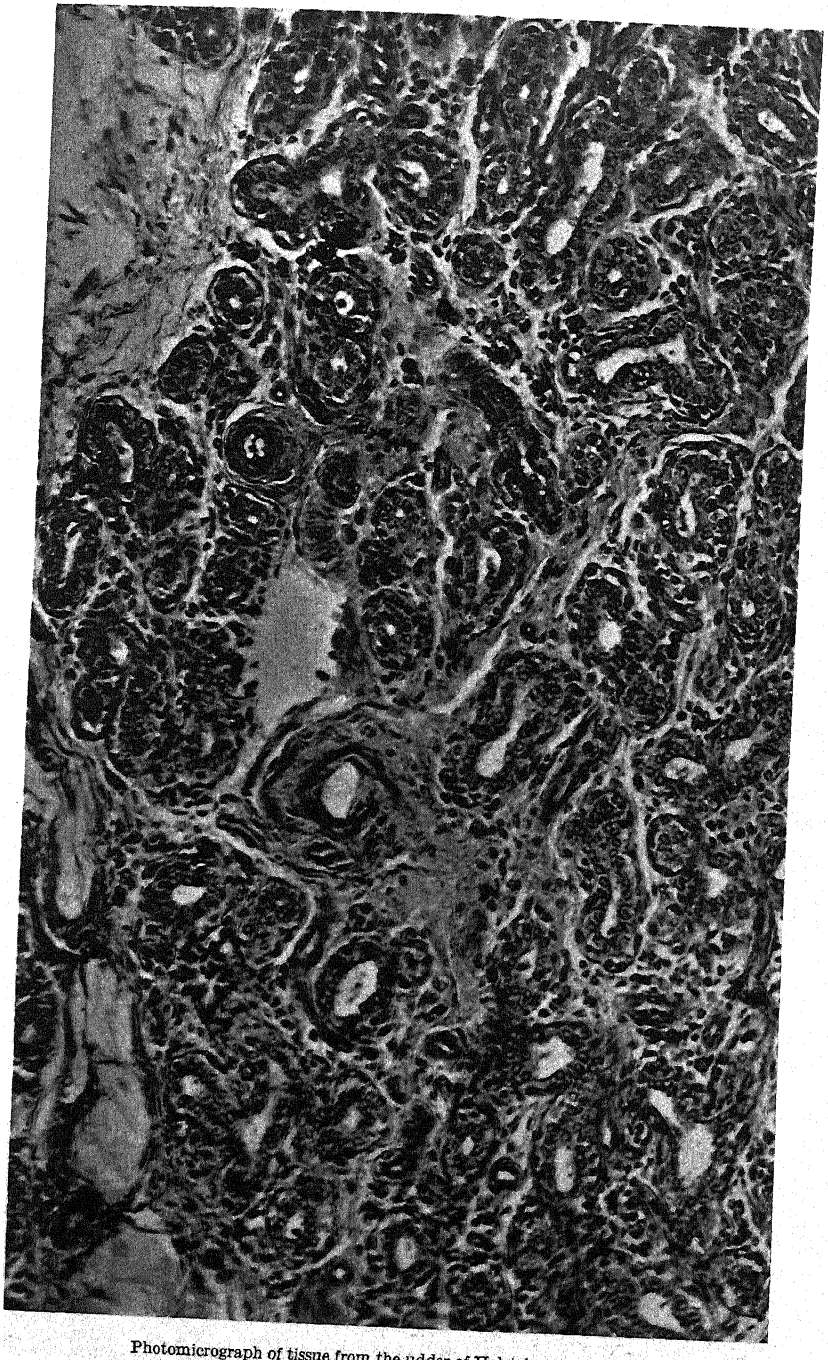
Vertical transverse section through a rear quarter of the udder of Holstein cow no. 299.



Photomicrograph of tissue from the udder of Holstein cow no. 299.



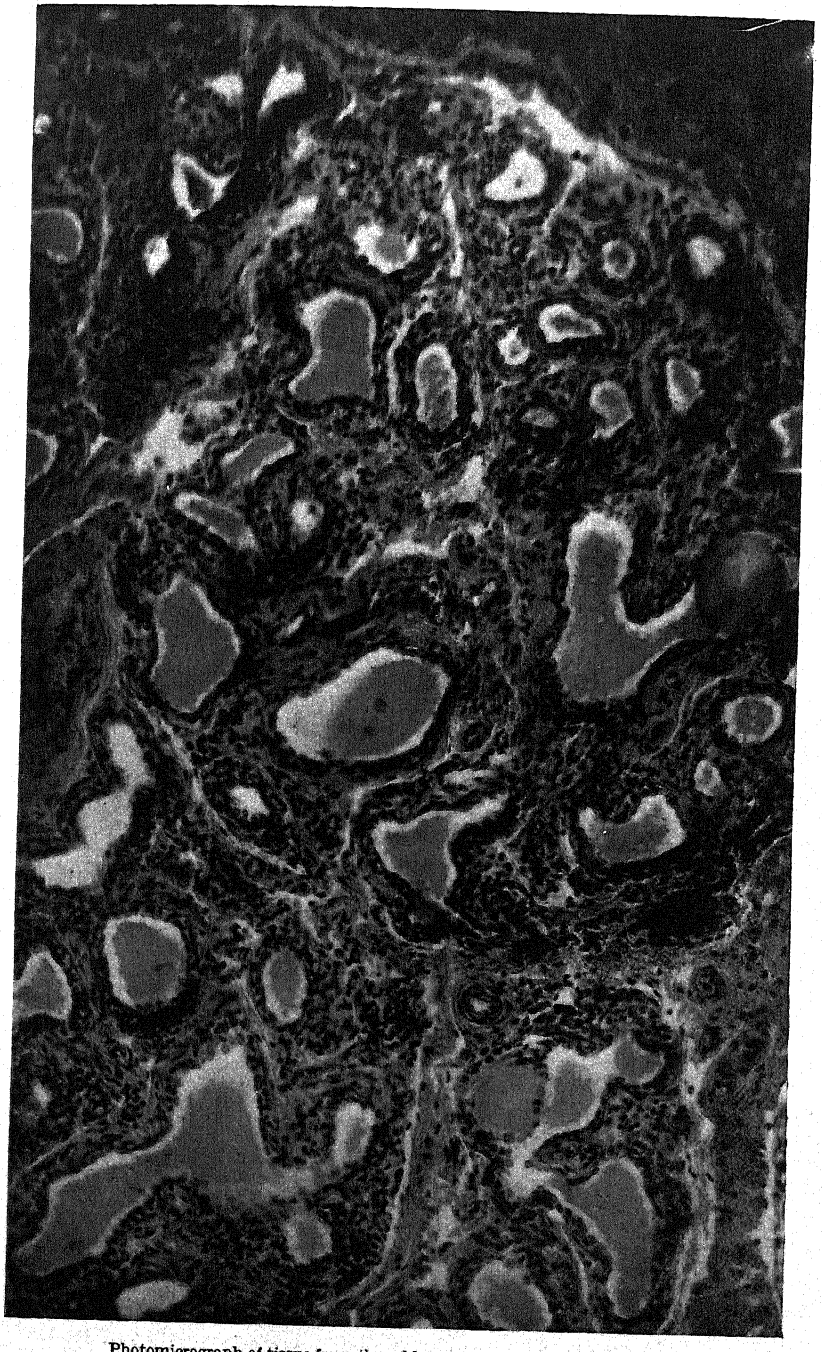
Vertical transverse section through a rear quarter of the udder of Holstein cow no. 827.



Photomicrograph of tissue from the udder of Holstein cow no. 827.



Vertical transverse section through a rear quarter of the udder of Aberdeen Angus cow A-300.



Photomicrograph of tissue from the udder of Aberdeen Angus cow A-300.

TABLE 12.—*Size and capacity of udder of Herefords, Holsteins, and Aberdeen Angus*

Item	Herefords									Holsteins			Aberdeen Angus
	Group 1 (dairy management)					Group 2 (beef management)				Average for both groups				
	H-2	H-5	H-6	H-16	Average	H-1	H-4	H-17	Average		No. 299	No. 827	Average	A-300
Size (empty weight) of udder---pounds--	9.95	7.15	8.35	14.50	9.99	6.30	6.35	18.00	10.22	10.09	30.50	22.00	26.25	44.00
Quantity of fluid held by 2 right quarters of udder----------cubic centimeters--	500	700	945	2,225	1,092.50	2,080	490	2,460	1,676.67	1,342.86	9,980	6,669	8,324.50	1,810
Milk equivalent capacity of secretory system of entire udder--------pounds--	2.27	3.18	4.30	10.11	4.97	9.45	2.23	11.18	7.62	6.10	45.36	30.31	37.84	8.23
Relation of capacity to weight of udder percent--	22.81	44.48	51.50	69.72	47.13	150.00	35.12	62.11	82.41	62.25	148.72	137.77	143.25	18.70
Stage of dryness: Time since last milked, months-days.	3-12	1-9	3-14	0-26	----------	0-18	----------	----------	----------	----------	0-22	7-12	----------	(1)

[1] About 25 months.

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at first that the comparatively high value for relation of capacity to weight of udder in the case of H–1 was to some extent the result of having nursed a calf until a short time before she was slaughtered. However, among the other Herefords the duration of the dry period did not differ greatly, and was nearly the same for H–5, H–16, and H–1, yet the capacity and relation of capacity to weight of udder for these three animals differed greatly. The dry periods of the Holsteins also varied from 22 days to more than 7 months, but their ratios of capacity to weight of udder were similar. Apparently variations in the duration of gland inactivity are at least only partly responsible for the differences in udder capacity or for the relation of capacity to weight of udder shown in this study, unless it be in the case of the Aberdeen Angus.

The fluid-holding capacity of the udder per unit of weight is consistently low for the beef cows studied, indicating that these udders contain a low quantity of secreting tissue in proportion to fat or connective tissue.

The number of animals is not sufficiently large to warrant the calculation of correlation coefficients but there appears to be a high positive relationship between empty weight, capacity, and ratio of capacity to weight of udder and milk production, except where the differences in production are so small as to be of no importance. In other words, there is a high positive correlation if the Holsteins, the highest producing Hereford, and the three lowest producing Herefords are considered as separate groups, but little if any correlation among the three lowest producing individual Herefords.

In view of the fact that the Holsteins produced 2,461 percent more milk and 1,681 percent more butterfat than the Herefords in group 1, the 163 percent greater empty weight of the Holstein udders, the 661 percent greater capacity of their udders, and the 204 percent greater capacity to weight ratio of their udders seem relatively small differences. These differences in udder size and capacity, however, were much more significant of the difference in producing capacity between the breeds than any of the udder characteristics studied on the live animals. This is in accordance with the results obtained in the study (*10*) of quality, size, capacity, gross anatomy, and histology of cow udders in relation to milk production in 11 registered and grade cows of dairy breeds, which showed that the only significant correlations between udder characteristics and producing capacity were for size and capacity of udder.

GROSS ANATOMY

The method of preparing and sectioning udders in making a comparative study of their gross anatomy was described in a previous publication (*4, Rept. 1929, p. 8*). In each case the right half of the udder was used in measuring capacity, and subsequently frozen, sectioned, and photographed. The left half was used for histological studies. The comparative size of the 10 udders used in this study is illustrated in plate 1, which shows a vertical transverse section through a rear quarter of each udder, all reduced to the same scale. Plates 2, 4, 6, 8, 10, 12, 14, 16, 18, and 20 show in greater detail the appearance of the gross anatomy of a vertical transverse section through a rear quarter, for the seven Herefords, the two Holsteins, and the Aberdeen Angus. A brief description of the gross anatomy is given under a

general discussion of the performance and udder characteristics of each cow.

HISTOLOGY [5]

The comparative histology of the tissues from the udders of the cows was studied according to the method described in a previous publication (*10*). The results obtained are reported as a part of the general discussion of the performance and udder characteristics of the individual cows. The histological appearance of the tissue from each udder is shown in plates 3, 5, 7, 9, 11, 13, 15, 17, 19, and 21.

UDDER CHARACTERISTICS AND PERFORMANCE OF EACH COW

HEREFORD COW H-2

Hereford cow H–2 was kept in the dairy herd and milked twice daily through one lactation period. Her total production for the entire lactation period of 153 days was only 126.00 pounds of milk and 6.52 pounds of butterfat. She had been dry for 3 months 12 days and pregnant for 157 days when slaughtered. The fetus weighed 3,561 grams (7.85 pounds). Ante-mortem examination showed that her udder, though extremely retarded in development and lacking in gland tissue, was of fair quality, and was more than medium in abundance of fiber; the openings from the teat canal to the cisterns were on the average very small and the free space was comparatively low. The udder was relatively low in weight, next to the lowest in capacity, and next to the lowest in relation of capacity to weight of udder.

Gross anatomy (pl. 2).—The gland tissue was very scanty, and limited to only a small proportion of the area of the sections. The sections through or close to the teats appeared to contain about one-third gland tissue; those near the front and rear of the udder contained little or none. The greater part of every section consisted of what appeared to be fat. The gland tissue was comparatively open in structure with a small but well circumscribed cistern. The openings to the cisterns were comparatively small. The ducts were well distributed and of medium size. Very little visible connective tissue was observed. The gland tissue was of medium firmness and the fat was very firm.

Histology.—Because of the extremely fatty condition of this udder, sections for histological study could be obtained only from areas in the lower portions of the front and rear quarters. This gland was found to be in the complete resting state with the exception of a few lobules here and there which showed some activity. This udder showed a rather heavy fibrous framework, and interlobular fat deposits were noted in the different areas examined. The areas illustrated in plate 3 were taken from a point near the base of the left rear quarter.

General.—There is no record of this cow having had any udder disturbance requiring treatment. The ante-mortem observations relative to abundance of fiber or connective tissue are supported by the histological findings, though the connective tissue is not readily visible in the gross sections. Liberal production could not be expected from an udder having the limited quantity of gland tissue indicated by the section illustrated.

[5] The histological studies were made by G. T. Creech, Pathological Division, Bureau of Animal Industry, U. S. Department of Agriculture.

HEREFORD COW H–5

Hereford cow H–5 was kept in the dairy herd and milked twice daily through a lactation period which continued for 202 days and yielded a total of 255.4 pounds of milk and 9.86 pounds of butterfat. She had been dry for 1 month 9 days and pregnant for 73 days when slaughtered. The fetus weighed 46 grams. Her udder was above the average in quality, yet extremely retarded in development and deficient in quantity of gland tissue. Ante-mortem examination indicated more than an average quantity of fiber, extremely small openings to the cisterns, and comparatively low free space. This udder was third from the lowest in empty weight and in capacity, and fourth from the lowest in relation of capacity to weight.

Gross anatomy (pl. 4).—Though the udder was very small, it consisted almost entirely of gland tissue with only small areas of fat in any of the sections except those at the extreme rear. In the rear quarter, especially in the lower half, the tissue was distinctly porous, and the cistern was of good size and somewhat divided. In the front quarter the cistern was not distinctly circumscribed but consisted of a honeycomb mass of ducts covering a relatively large part of the area above the teat. The opening between the teat and the cistern was of good size in the rear quarter and even larger in the front quarter. The amount of visible connective tissue was not great and it was comparatively finely divided. Both the gland tissue and the fat were firm at the time the sections were photographed.

Histology.—The histological examination showed this udder to be in the resting state. Sections were obtained from only three locations. Areas of round cell infiltration were noted in this gland, and in certain areas there appeared to be some increase in the interlobular and intralobular connective tissue. Large areas of fat as well as smaller interlobular fat deposits were observed in this udder. The areas illustrated in plate 5 were taken from a point in the upper portion of the left rear quarter.

General.—There is no record of this cow having had any udder disturbances requiring treatment. The ante-mortem observations indicated a greater quantity of fiber than was observed in the gross anatomy sections. Though excessive fat deposition was not indicated by ante-mortem examination or by the gross sections the histological studies indicated that considerable deposition had taken place. Though the proportion of gland tissue to fat was high, the quantity of gland tissue was so small that liberal production of milk could hardly have been possible.

HEREFORD COW H–6

Hereford cow H–6 was kept in the dairy herd and milked twice daily through a lactation period which, with much difficulty, was kept active for 159 days, and which resulted in a total yield of only 80.6 pounds of milk and 3.73 pounds of butterfat—an average daily milk production of 0.51 pound. This is the poorest production for any cow in this group. She had been dry for 3 months 14 days and pregnant for 91 days when slaughtered. The fetus weighed 192 grams. The udder was one of the lowest in quality in this group. It was extremely retarded in development, and very deficient in quantity of gland tissue. The fiber was of average abundance, the openings to cisterns were very small, and the free space was comparatively low

The udder was fourth from the lowest in empty weight and in capacity, and fifth from the lowest in relation of capacity to weight.

Gross anatomy (pl. 6).—The gland tissue in this udder could not readily be distinguished from the fat. There was a layer of firm fat across the upper surface, but the rest was soft and lacking in outline pattern and color differentiation. Incomplete freezing of the udder when sectioned may have resulted in a tendency for the saw to tear the tissue, leaving the surface rough, but it could not have been entirely responsible for the lack of differentiation in color in the gross sections of this udder. Apparently the gland tissue was limited in quantity and interspersed with fat. The cisterns in both front and rear quarters were small, round, and tubular, and little more than continuations of the teat canal, though the cistern in the rear quarter was somewhat divided at its upper extremity. The openings from the teat canal to the cistern in both front and rear quarters were much larger than the ante-mortem grades would indicate. Aside from a small area in the immediate vicinity of the cisterns, the tissue was dense and almost devoid of visible ducts. An unusually large quantity of visible connective tissue was present, although only an average quantity was indicated by the ante-mortem examination.

Histology.—This udder was small and very fat; consequently sections were obtained only from areas in the lower part of the front and rear quarters. This gland was in the resting state and showed little deviation from the normal, other than the large amount of fat which appeared, not only as interlobular deposits, but as rather large areas of fat here and there, in places ordinarily occupied by normal gland tissue. The area illustrated in plate 7 was taken from near the center of the left front quarter.

General.—Both the gross anatomical and histological studies indicate a marked lack of secretory tissue and an excess of fat, though the fatty condition was not noted at the time of ante-mortem examination. As shown in table 4, this cow was milked until she was producing an average of only 0.17 pound of milk daily. The average production for the first 10 days in milk was only 1.34 pounds, and the highest for any one day in the lactation period was only 2.4 pounds. Again it appears that failure to function was due to extreme deficiency in secretory tissue in the udder.

HEREFORD COW H–16

Hereford cow H–16 was kept in the dairy herd and milked twice daily through a lactation period which continued for 329 days and yielded 1,767.8 pounds of milk and 90.52 pounds of butterfat. Her production and persistency were far superior to those of any of the other Herefords for which production records were obtained. She had been dry for 26 days and pregnant for 223 days when slaughtered. The fetus weighed 17,464 grams (38.5 pounds). Ante-mortem examination showed that her udder was near the average for the group in quality, the fiber was of medium abundance, the openings to the cisterns were very small, and the free space was high. Though this udder was small and very deficient in gland tissue when compared with those of dairy cows, it exceeded in dimensions that of any other Hereford in the dairy herd. The empty weight of udder was higher than that of the other Herefords having production records, but ranked fifth from the highest in the entire group of cows compared in this

study. The udder was fourth from the highest in the entire group in capacity, and in relation of capacity to weight of udder.

Gross anatomy (pl. 8).—Though the quantity of gland tissue was greater than that of any of the other Herefords in the dairy herd, it occupied only about one-half to two-thirds of the area of sections through the teats and very little or none in sections from other parts of the udder. The outline of the gland tissue, however, was distinct and readily distinguishable from the areas of fat above and around it. The cistern in the rear quarter was small, divided, and appeared to be a continuation of the teat canal; the one in the front quarter was similar except that it was more tubular and less divided. The tissue was fairly firm and inclined to be dense except in areas close to the cisterns. The sections contained comparatively large quantities of visible connective tissue which was uniformly distributed, although the ante-mortem examination indicated only an average quantity of fiber. The areas of fat were of moderate firmness. The openings from the teat canals to the cisterns were considerably larger than the ante-mortem grades would indicate, and the cisterns were smaller than the grades for free space would suggest.

Histology.—This was a small, fatty udder. Owing to the large amount of fat present it was possible to section only an area from the lower portion of the rear quarter for histological study. The gland was found to be in the resting state. There was a limited amount of round cell and leucocytic infiltration. The areas sectioned and illustrated in plate 9 showed a considerable amount of fat, both interlobular and in areas ordinarily occupied by gland lobules, and were taken from a point near the base of the left rear quarter.

General.—This cow was somewhat older, larger, and better developed than the others already discussed. Although her udder was described as small and deficient in gland tissue, its development and its capacity for production were superior to those of the other three Herefords kept in the dairy herd.

HEREFORD COW H–1

Hereford cow H–1 was kept in the beef herd and allowed to nurse her calf from December 14, 1928, until late in May 1929. She was not pregnant when slaughtered. Though the udder was very small and very deficient in glandular development it was about medium in quality. According to ante-mortem observations the fiber was abundant, and above medium in coarseness and harshness; the openings from teat canal to cisterns were variable, being graded as medium in the right front and very small in the right rear quarter; and the free space was about average. In empty weight this udder was the smallest in the entire group, in capacity it was fifth from the highest, and in relation of capacity to weight of udder it was higher than for any other cow in any of the breeds compared in this study.

Gross anatomy (pl. 10).—Though the udder was the smallest in the group it was made up almost entirely of gland tissue. There were practically no visible areas of fat in any of the sections except those at the extreme rear of the udder. This is in agreement with the high grade for "nearness to surface" (table 11). The udder was not completely frozen when sectioned, but the cuts were fairly clean and the surfaces of the sections were smooth. In the front quarter the cistern was of medium size, definitely circumscribed, and the opening into it

from the teat canal was large. In the rear quarter the cistern was very small, somewhat divided, and the opening into it was much smaller than in the front quarter. The size of openings into cisterns corresponds rather well with the grades assigned on ante-mortem examination, though the grades for free space do not appear to be related closely to the size of the cisterns. Only a moderate amount of connective tissue was visible in the gross sections although the ante-mortem examination indicated an abundance of fiber. The tissue was comparatively dense, the ducts being limited almost entirely to areas close to the cisterns and to the lower half of each section. The tissue was described as a trifle soft but more like the secretory tissue of a dairy cow than that of any of the other Herefords.

Histology.—One of the sections, from the lower portion of the rear quarter (pl. 11, *B*), showed this particular area of the gland to be in a complete resting state. Sections from other areas (pl. 11, *A*) showed evidence of considerable glandular activity. Many of the alveoli were filled with free cells containing fat droplets, giving the various lobules as a whole a peculiar fatty appearance in some sections. Interlobular fat deposits were seen in some of the sections.

General.—The gross sections consisted almost entirely of gland tissue, although the actual quantity was very small. The shortness of the dry period preceding slaughter may have been partly responsible for the close resemblance of the tissue in this udder to that of a dairy cow. Moreover, this cow was in a poor condition of flesh, which may have affected the amount of deposition of fat in the udder.

HEREFORD COW H–4

Hereford cow H–4 was kept in the beef herd. She aborted December 20, 1928; and information is not available as to whether she was nursed by the calves of other cows, or the possible effect of such nursing on the length of the lactation period and the condition of the mammary gland. She was pregnant when slaughtered, but the date of breeding was not known. The fetus weighed 236 grams. The udder appeared on ante-mortem examination to be the smallest in the entire group, and its empty weight was within 0.05 pound of the lowest recorded. The glandular development was extremely deficient, the quantity and proportions closely approximating those of a 6-month-old Holstein calf. The udder quality was far below the average for the group of udders compared in this study. The fiber was of medium abundance and coarseness. The openings from the teat canals to cisterns appeared on ante-mortem examination to be extremely small and the free space was one of the two lowest in the group. This udder was not only next to the lowest in empty weight, but it was the lowest of all in capacity, and third from the lowest in relation of capacity to weight.

Gross anatomy (pl. 12).—Not only was the udder extremely small, but it contained a comparatively large proportion of fat, thereby supporting the grade assigned for "nearness to surface" (table 11). The gland tissue did not occupy more than two-thirds of the area of any section, and in most sections it did not occupy more than one-half of the area. In the rear quarter the cistern was very small and the opening from the teat canal was extremely small. In the front quarter also the cistern was very small, but the opening from the teat canal was very slightly larger than in the rear. These findings are

generally in accord with ante-mortem observations on size of openings and amount of free space. The gland tissue was not definite in outline and not easily distinguishable from the fat. Except for a small area close to the cistern the ducts were small and very few in number. It appeared that a fairly large quantity of connective tissue was present in this udder and that the gland tissue was interspersed with fat. The gland tissue was inclined to be soft, but the surrounding fatty areas were very firm.

Histology.—This udder was very small and extremely fat, and sections could be obtained only from areas near the center of the front quarters and the lower part of the rear quarter. This gland was in the resting state and showed little evidence of functional activity. The outstanding histological finding in this udder was the excessive amount of fat present and intermixed with the glandular tissue. There were not only extensive interlobular fat deposits, but in places there were areas the size of several lobules occupied by fat, instead of the normal gland tissue. The area illustrated in plate 13 was taken from near the center of the left front quarter.

General.—This udder probably had the least grandular development of any compared in this study, though in some respects that of H–2 was not greatly different. The gross anatomical findings support the ante-mortem observations unusually well for abundance of gland tissue and its closeness to the surface, abundance of fiber, and size of cistern openings. Uncertainty as to the duration of lactation and the possible effect of abortion on mammary activity and development make conclusions difficult. It appears, however, that this cow did not possess the necessary development for liberal lactation.

HEREFORD COW H–17

Hereford cow H–17 was kept in the beef herd following an abortion on June 12, 1928. As in the case of H–1, information is lacking as to her lactating capacity and as to whether she was nursed by the calves of other cows. Moreover she was in advanced pregnancy (271 days) when slaughtered and her udder was "making up" and therefore not comparable to the others in regard to many characteristics. The fetus weighed 27,443 grams (60.5 pounds). Though small and deficient in gland tissue in comparison with that of a dairy cow, the udder appeared to be superior to that of any of the other Herefords at the time the ante-mortem observations were made. In quality, however, it was well below the average for the group of cows compared. The fiber was of medium abundance, coarseness, and harshness. The openings from the teat canals to the cisterns appeared on ante-mortem examination to be larger on an average than for any other cow in the group, the right front one being very small but the right rear one large. The amount of free space appeared to be slightly below medium. In empty weight the udder ranked fourth from the highest, in capacity it was third from the highest, and in relation of capacity to weight it was fifth from the highest.

Gross anatomy (pl. 14).—Though this udder was of fairly good size, a large proportion of the area of the sections consisted of fat. In the sections carrying the teats only about 50 to 60 percent of the total area consisted of gland tissue; in the sections near the front and rear of the udder there was little or no gland tissue. The outline of the gland tissue was fairly distinct, though in many sections there was an

area surrounding it which seemed to contain a large proportion of connective tissue and to be intermediate between the gland tissue and the fat. The udder was not well frozen when sectioned, but the surfaces of the sections were fairly smooth. In the rear quarter the cistern was of moderate size but the front cistern was large. Both cisterns were divided. Though the ante-mortem examination indicated a rather marked difference between the front and rear quarters in size of opening from teat canal to cistern, the gross sections showed that both were comparatively large. The tissue was of more than average openness, especially in the vicinity of the cisterns. A medium quantity of connective tissue was visible in the sections though it was rather indistinct in outline. The tissue itself was described as mushy but the areas of fat were of medium firmness.

Histology.—This udder showed glandular activity in all the areas examined, with indications here and there of the gland being partly in the resting state. A few small areas of round cell infiltration were noted, but for the most part this gland was normal in appearance. The fibrous connective tissue of this udder had the appearance of being of a soft or loose texture. The area illustrated in plate 15 was taken from the upper portion of the left front quarter.

General.—The ante-mortem observations are usually well supported by the gross-anatomical findings for most of the items compared. The quantity of gland tissue appeared to be nearly the same as for H–16, the Hereford kept in the dairy herd, which closely approximated H–17 in age and in size. It would appear that the advanced stage of gestation was largely responsible for the indications of activity reported in the histological study, and to some extent for the size of the udder and the quantity of gland tissue present.

HOLSTEIN COW NO. 299

Cow 299, a registered Holstein, was bred and raised in the Bureau's dairy herd at Beltsville, Md. Her production record made on three milkings daily at 3 years 3 months of age was 14,295 pounds of milk and 463 pounds of butterfat. She had been dry for 22 days at the time of slaughter, and was not pregnant. She was slaughtered because of a relaxed condition of the sacroiliac joint. Ante-mortem measurements in regions of the body likely to be affected by this condition were taken on the uninjured side. Though some of the carcass measurements may have been slightly affected, the resulting error is believed to be of little importance. On ante-mortem examination the quantity of mammary-gland tissue appeared to be small. The quality was very high (the highest in the entire group compared in this study) and the udder covering was extremely loose. The fiber was comparatively abundant, rather finely divided, and of medium harshness, and the free space was next to the highest in the entire group. Grades were not assigned to the size of the openings into the cisterns. Though not a large udder, its empty weight was next to the highest, its capacity was the highest, and its relation of capacity to weight was next to the highest in the entire group of udders compared.

Gross anatomy (pl. 16).—Although the udder was slightly thawed on the surface, the saw cuts were fairly smooth. Aside from a very small area at the extreme top, practically the entire surface of each section consisted of gland tissue. The rear cistern was very small

and the front one was almost lacking. There seems to be no relation between size of cistern and amount of free space in this udder. The cisterns were smaller than the teat canals. The tissue was of medium density, inclined to be wet but had a fairly good body, and contained a large proportion of clearly visible connective tissue of medium coarseness. The openings into the cisterns were of medium size.

Histology.—This was considered a nonactive or dry udder, and with the exception of a number of scattered lobules the histological examination showed the gland to be largely in the resting state. Round cell infiltration was noted in all of the sections and a limited number of the alveoli contained pus cells. A number of milk concretions were seen in some of the sections. The area illustrated in plate 17 was taken from the lower part of the left front quarter.

General.—The records show that at one time there had been inflammation in the right rear quarter, but the udder appeared to be sound when examined before slaughter. Post-mortem examination failed to show any indications that this inflammation had been present. The abundance of tissue was rated as low on ante-mortem examination but appeared to be fairly abundant in the gross sections. Judgment as to abundance of fiber and presence of fat in the udder before death is rather well supported by gross anatomical findings.

HOLSTEIN COW NO. 827

Cow 827, a registered Holstein, was bred and raised in the dairy herd at the Beltsville Experiment Station. Her production record made on three milkings daily at 2 years 7 months of age was 14,257 pounds of milk and 522 pounds of butterfat. She had been dry for 7 months 12 days at the time of slaughter, and was not pregnant. On ante-mortem examination the udder showed only a very small quantity of gland tissue, indicating a high degree of involution since the udder went dry. In quality this udder ranked second from the highest in the group compared in this study. The fiber appeared to be above medium in abundance, and medium in coarseness. The openings from teat canals to cisterns appeared to be small in front and medium in the rear. The free space was the highest in the entire group, though there were no indications of large cisterns. Though this udder was small in size, its empty weight was third, its capacity was second, and its relation of capacity to weight was third from the highest in the entire group of udders compared.

Gross anatomy (see pl. 18).—A considerable quantity of fat was present in all sections. In the sections carrying the front and rear teats and in other sections through the middle part of the udder there were fairly large areas of fat near the top, and a strip along the periphery—under the skin—which apparently consisted chiefly of a loosely formed fibrous structure, giving the appearance of the skin breaking away from the gland tissue. This suggests the possibility that a pocket of formalin may have been formed in this area at the time of filling, and that the capacity of the udder as recorded may be a trifle high. It is worthy of note, however, that the histological study showed large replacements of gland tissue with fat near the periphery. There was no distinctly circumscribed cistern in either the front or rear quarter, though numerous medium-sized ducts were present in the area usually occupied by the cistern. The cisterns

were small in total volume and very much divided. The opening from teat canal to cistern was about average in the rear quarter and slightly larger in the front quarter; and both openings were larger in diameter than the cisterns themselves. The tissue was low in porosity, about average in firmness, wet, and the visible connective tissue was abundant. The tissue appeared to be found in all parts.

Histology.—This was a small, nonlactating udder, with considerable fat. Histologically the general structure of the udder showed little deviation from the normal. The glandular portion was found to be in the resting state. Practically all the lobules showed the deposition of fat to a greater or less extent, and some of the lobules, toward the periphery of the organ, were largely replaced by fat. The udder showed a rather heavy fibrous structure throughout, with wide bands of fibrous tissue separating many of the lobules. The area illustrated in plate 19 was taken from the upper portion of the left rear quarter.

General.—There is no record of this cow having had any udder disturbance requiring treatment. Ante-mortem judgment as to abundance of fiber is well supported by both gross anatomical and histological findings. The free space did not bear a close relation to size of cisterns, but the size of cisterns was found to be much as described on ante-mortem examination. The ante-mortem observations would indicate a smaller quantity of fat than was found in both the gross and histological sections.

ABERDEEN ANGUS COW A-300

Cow A-300, a registered Aberdeen Angus had been a persistent breeder and a consistent winner in the show ring for a number of years, but nothing is known of her milk-producing ability. She had been nonlactating for about 25 months when slaughtered, and was not pregnant. Ante-mortem examination of the udder indicated a quality close to the medium, a very loose covering, a medium quantity of gland tissue, a comparatively small quantity of rather coarse fiber, and slightly more than average free space. The udder was described as fatty. Grades were not assigned to indicate the size of openings between the teat canals and the cisterns. Though only medium in size it was the highest in the entire group in empty weight; sixth from the highest in capacity, and the lowest of all in relation of capacity to weight, indicating that the udder consisted largely of material other than gland tissue.

Gross anatomy (pl. 20).—Aside from a small area above the rear teat, practically the entire udder consisted of fat. In the front quarter the gland tissue was little more than sufficient to enclose the duct or cistern, which was not more than a quarter of an inch in diameter, horizontal in position, and approached the teat canal from the rear. The rear quarter contained an area of gland tissue of irregular shape, about 5½ inches at its maximum height, about 3 inches at its maximum width, and surrounded laterally and superiorly with solid fat. The rear cistern was very small and somewhat divided. The front one was almost entirely lacking. The opening from teat canal to cistern was only moderate in size in the rear and extremely small in the front quarter. There were only a few small ducts except in the immediate vicinity of the cistern. A fairly liberal quantity of comparatively finely divided connective tissue was visible. The sections were firm when kept at low temperatures, but, owing

to the very great proportion of fat, became soft at higher temperatures. The fatty areas as well as the areas of gland tissue contained a considerable quantity of connective tissue or fiber. As the fatty areas receded these stood out in relief, giving the sections a rough appearance, particularly in the photographs.

Histology.—This udder showed an extreme fatty condition in the gross specimen and it was possible to obtain sections from only one area in the lower portion of the rear quarter. Histologically this area was found to be in the complete resting state and quite fibrous. The fibrous condition may have been due in part to the advanced age of the animal. A number of milk concretions were noted in the section. (See pl. 21.)

General.—Undoubtedly this udder had the largest proportion of fat to gland tissue of any udder yet studied. In making the antemortem observations the udder was described as fatty but the condition was underestimated. Probably because of the extreme fatty condition the quantity of fiber as shown by both gross and histological sections was also underestimated. The gross anatomical and histological findings are in very close agreement. The high condition of this cow and the 25 months dry period probably account for the large amount of fat in the udder, but the extremely small quantity of gland tissue would appear to have made it impossible for her to secrete any considerable amount of milk.

DISCUSSION AND SUMMARY

The milk- and butterfat-producing ability of four Hereford cows maintained under dairy-herd conditions is compared with that of two registered Holstein cows. A comparison is also made of the weight and measurements of the body, the weight and measurements of the internal organs and body parts, and the mammary-gland development and udder structure of the four Hereford cows, the two registered Holstein cows, three Hereford cows kept under typical beef-herd conditions, and an aged Aberdeen Angus cow.

The average production of the four Hereford cows (that were accustomed to conditions of dairy-herd management prior to their first calving and milked and fed under the same environmental conditions as the dairy herd) was 557.45 pounds of milk, testing 4.96 percent butterfat and containing a total of 27.66 pounds of butterfat. The average lactation period was 211 days. The average production of the two Holstein cows was 14,276 pounds of milk, testing 3.45 percent butterfat and containing 492.5 pounds of butterfat. The average lactation period was 364 days. The production of the two Holstein cows was 2,461 percent greater in milk and 1,681 percent greater in butterfat than the average production of the four Hereford cows.

Although the four Herefords milked in the dairy barn were considerably undersized, they could hardly have failed to make a more creditable showing, considering their opportunities both before and during lactation, if their inheritance had included the capacity for abundant lactation or the stimulus to continue lactation over an extended period. Apparently they did not possess these qualities.

In live weight and in every body dimension measured before slaughter, the Herefords studied were smaller than either the Holsteins

or the Aberdeen Angus. Though heavier, the Aberdeen Angus was smaller than the Holsteins in the measurements of length and height, but greater in some other measurements, particularly those of width and circumference of body that are affected to the greatest extent by fleshing. In this connection, attention is called again to the possible effect of age differences on the comparisons of absolute values of weight or measurements for different breeds.

When the ante-mortem data are compared on the basis of ratios based on height at withers or on length of head, a marked similarity is shown in the body proportions of the Holsteins and the Herefords. The ratio for width of fore chest appears to be the outstanding exception. For the measurements of height and length, which indicate "scale", the body proportions of the animals in all three breeds are similar. The outstanding differences in body proportions are associated with those measurements, such as width and circumference of barrel, that are most directly affected by deposition of fat. This supports the finding of a previous investigation that the cows of different breeds are generally similar in skeletal structure—the differences in type, resulting from breeding and selection, being due primarily to the degree of fleshing.

Although the contour areas for fore chest and paunch increased progressively in size from Hereford to Holstein to Aberdeen Angus and there was considerable difference in shape of the contours, only a slight difference existed in the proportion above and below the vertical midpoint.

The body surface area was almost the same for Holsteins and Aberdeen Angus. That for the Herefords was much less.

The volume of barrel also was nearly the same for Holsteins and Aberdeen Angus. That for the Herefords was very much less.

The thoracic index decreased with considerable regularity from Herefords to Holsteins to Aberdeen Angus, indicating that the Holsteins were intermediate and the Aberdeen Angus was the highest in proportion of width to depth of fore chest.

Abdominal indexes were nearly the same for all three breeds.

The legginess was almost the same for the Holsteins as for the Herefords, but much lower for the Aberdeen Angus.

Great differences existed in wedge shape. The Herefords had very little vertical wedge shape, the Holsteins had more than 10 times as much as the Herefords, and the Aberdeen Angus had more than three times as much as the Holsteins. In lateral wedge shape there was a marked decrease from Herefords to Holsteins to Aberdeen Angus. In circumference the wedge shape was almost the same for the three breeds; and on the basis of contour areas the Herefords and the Holsteins were almost the same, but the Aberdeen Angus was distinctly less. The Aberdeen Angus was very wide in the fore chest as compared with the animals in the other breeds. Again the influence of fleshing—especially in the region of the fore chest—is shown in the values for wedge shape.

With few exceptions the post-mortem data, expressed in actual units of weight or measurement, are distinctly lower for the Herefords than for the Holsteins—the most marked differences being in the visceral fats, the endocrine glands, and the udder. A comparison of the Aberdeen Angus with the Herefords shows relationships that are similar to the Holstein-Hereford relationships in most respects.

However, in comparing the Aberdeen Angus with the Holsteins, although the four greatest differences are in weights of adrenals, udder, kidneys, and thyroid, all of which are greater for the Aberdeen Angus, the data for the Aberdeen Angus are actually lower than for the Holsteins for more than two-thirds of the items.

When considered in relation to the empty body weight of the animal, there was a definite tendency for most of the weights and measurements of the internal organs and body parts to be greater for the Herefords than for either the Holsteins or the Aberdeen Angus and greater for the Holsteins than for the Aberdeen Angus, except for the visceral fats, the udder, the ovaries, the kidneys, and some of the endocrine glands.

When grouped on the basis of anatomy and function, it appears that the organs and parts of both the Holsteins and Aberdeen Angus averaged greater in actual magnitude than those of the Herefords, while those of the Aberdeen Angus averaged smaller than those of the Holsteins in the circulation and respiration group and in the digestion group. In proportion to their empty body weight, however, the Herefords had larger organs than the Holsteins in all except the endocrine gland and visceral fats groups, and larger organs than the Aberdeen Angus in all except the urogenital, endocrine gland, and visceral fats groups. The Holsteins had larger organs than the Aberdeen Angus in all except the urogenital and endocrine gland groups. The effect of fleshing is clearly indicated, for as it increases the total animal structure, the organs become proportionately smaller. The only group consistently overcoming this factor, showing greater average values for Holsteins than for Herefords and greater values for the Aberdeen Angus than for either Herefords or Holsteins, was the one consisting of endocrine glands.

Despite the breed differences in size of internal organs and body parts, there was very little difference in dressing out percentage between the Herefords and the Holsteins. The dressed weight was 50.0 percent of the live weight obtained immediately before slaughter for the four Herefords in group 1 (dairy management), 49.9 percent for the three Herefords in group 2 (beef management) and 54.7 percent for the two Holsteins. When based on empty body weight instead of live weight, the dressing out percentages were 60.2, 60.1, and 62.5, respectively. Owing to the difference in procedure incidental to the preservation of her skeleton, the carcass weight of the Aberdeen Angus cow could not be determined.

Five of the seven Herefords were extremely deficient in mammary-gland development. The other two, though more advanced, still were distinctly deficient. The greater udder development of H–16 and H–17 as compared with the other Herefords is shown both by the photographs of the living animals (figs. 1 and 2) and by the photographs of the gross sections of the udders (pl. 1). This difference may be due partly to their greater size and presumably more advanced age and partly to the fact that they came from a different line of breeding and had a different inheritance. Only two Herefords, one that nursed her calf until 18 days before slaughter, and one in advanced pregnancy, showed signs of activity in the gland tissue, and all except these two showed large quantities of fat deposits in the histological sections. The udder of the Aberdeen Angus was much larger and appeared to have a greater quantity of gland tissue, but on post-

mortem examination it proved to consist chiefly of fat. The Holsteins had udders of comparatively small size for the breed.

The Holsteins ranked considerably above the Herefords in most of the udder characteristics associated with "udder quality". The Aberdeen Angus ranked close to the Herefords in most of these items but above the Holsteins in a few. There was little difference between the breeds in those items that might be considered antagonistic to quality. Grades for milk veins and milk wells were in most cases definitely higher for the Holsteins than for the other breeds. Though the grades for quality of udder are in most cases higher for the Holsteins than for either the Herefords or the Aberdeen Angus, the differences do not approach the very great difference in producing capacity that actually existed between the Holsteins and the Herefords, and that in all probability existed between the Holsteins and the Aberdeen Angus.

In size (empty weight) of udder the Herefords in group 2 (beef management) differed from the Herefords in group 1 (dairy management) by only 2.30 percent; the Holsteins were 160.16 percent greater than the Herefords; and the Aberdeen Angus was 336.08 percent greater than the Herefords and 67.62 percent greater than the Holsteins. The comparatively high weight of udder in the Aberdeen Angus was due to excessive deposition of fat.

In capacity of udder the Herefords in group 2 (beef management) averaged 53.32 percent more than the Herefords in group 1 (dairy management). This may indicate a possibility that the Herefords in group 2 produced more abundantly than those in group 1. Actual milk production records for the cows in group 2 are not available, of course, but there is nothing in the appearance of the gross sections of the udders to indicate they had a greater quantity of mammary-gland tissue. The udder capacity of the Holsteins was 520.33 percent greater than the average for the Herefords, and 359.78 percent greater than for the Aberdeen Angus. The capacity of the Aberdeen Angus, however, was 34.92 percent greater than the average for the Herefords. The proportion of mammary-gland tissue in the udder of the Aberdeen Angus was extremely small. The low udder capacity of cows of the beef breeds studied was shown consistently.

In "relation of capacity to weight" of udder, the Herefords in group 2 (beef management) averaged 74.86 percent higher than those in group 1 (dairy management). The Holsteins exceeded the Herefords by 130.12 percent, but the Aberdeen Angus was 69.96 percent lower than the Herefords and 86.95 percent lower than the Holsteins. The low fluid-holding capacity per unit of weight for the beef-cow udders indicates a small quantity of gland tissue in proportion to fat or connective tissue. This was particularly true of the Aberdeen Angus, which had the heaviest udder with a capacity only slightly above the average for the Herefords, and the lowest relation of capacity to weight of all the udders included in this study. The effect of heavy fat deposition in the udder of this cow is clearly indicated, but the extent to which her long dry period was responsible for it is not definitely known.

For the six cows in this study having records of production there appears to be a high positive correlation between size (empty weight), capacity, and ratio of capacity to weight of udder and the quantity of milk produced, if the two Holsteins, the highest producing Hereford,

and the three lowest producing Herefords are considered as groups, but little if any correlation among the three lowest producing individual Herefords. Though the Holstein udders exceeded the Hereford udders by 163 percent in empty weight, 661 percent in capacity, and 204 percent in ratio of capacity to weight, these differences do not approach the corresponding differences of 2,461 percent and 1,681 percent, respectively, for milk and butterfat production. At the same time the percentage differences for udder size and capacity were much more significant of the producing ability than were the udder characteristics studied on the living animals. This is in harmony with previous studied, which showed size and capacity of udder were the only items of those studied that had significant correlations with records of production.

With few exceptions the antemortem observations were fairly well supported by the findings reported in the gross anatomical and histological studies, and the gross anatomical observations are perhaps even more closely supported by the histological findings.

The proportion of fat in the udder of the Aberdeen Angus was extremely high. The possible effect of prolonged inactivity on fat deposition has been discussed. In the Herefords it was high but less extreme. One of the Holstein udders had practically no visible fat in the gross sections, the other a moderate quantity. Though in a few instances the proportion of visible fat in the udders of the Herefords was comparatively small, the total quantity of gland tissue and the measured capacity of the udder were both so small that it is inconceivable they could have produced milk in abundant quantities. To this extent the findings with Herefords support the observations and conclusions previously reported for the Aberdeen Angus, which were to the effect that cows of the specialized beef breeds do not inherit a mammary development sufficient to enable them to be liberal milk producers.

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GROWTH AND DEVELOPMENT OF DICTYOSTELIUM DISCOIDEUM WITH DIFFERENT BACTERIAL ASSOCIATES [1]

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INTRODUCTION

The genus *Dictyostelium* and the group to which it belongs have been known since Brefeld (*2*) [3] described *Dictyostelium mucoroides* in 1869. Additional species have been added to the genus by Van Tieghem (*22*), Marchal (*10*), Olive (*13*), and Raper (*18*).

The developmental history of species of the Dictyosteliaceae is well known from the works of Brefeld (*2, 3*), Van Tieghem (*22, 23*), Olive (*14*), Harper (*6, 7, 8*), and Raper (*18*). Their investigations dealt either with the taxonomy of the group or with the formation of fruiting structures in certain species of the family, whereas little study was given to the vegetative stage and no attention was paid to the role of the bacteria that accompanied the slime molds. Some investigations of a physiological nature, however, were made by Nadson (*12*), Potts (*17*), Vuillemin (*24*), Pinoy (*15, 16*), and Skupienski (*20, 21*); and in their studies the role of the accompanying bacteria rightfully occupied the center of attention.

The relation between species of the Dictyosteliaceae and the bacteria with which they are associated has been a subject of dispute. Although any effective study of this relationship obviously would have to be made with the slime molds growing in association with pure cultures of bacteria of known identity, only a part of the studies previously reported were made with such cultures. Further, there is a disparity in the results reported by different investigators working with the same species of *Dictyostelium* in association with the same species of bacteria.

Because of the conflicting results reported by earlier students of the Dictyosteliaceae and because of the limited scope of their investigations, the need for additional study of the whole question of the relationship between species of this family and the bacteria that accompany them became apparent. The writer, therefore, undertook the investigation of this problem with the following objectives in view: (1) To study the range of different species of bacteria in

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[2] The author is particularly indebted to Prof. William H. Weston, Jr., of Harvard University, under whose direction the present studies were pursued, for valuable counsel and criticism during the progress of the work. The writer is also grateful to N. R. Smith, Division of Soil Microbiology, Bureau of Plant Industry, U. S. Department of Agriculture, for helpful advice, for identifying cultures of bacteria, and for generously supplying the greater portion of the bacterial cultures studied during this investigation; and to Dr. Charles Thom, in charge of the Division, for having originally suggested an investigation of this nature and for guidance during earlier work which forms a background for the present studies.

[3] Reference is made by number (italic) to Literature Cited, p. 314.

association with which a representative member of the Dictyosteliaceae could grow; (2) to study the manner in which myxamoebae of such a species feed upon bacteria; and (3) to study the relation between a species of the Dictyosteliaceae and the bacteria associated with it.

Dictyostelium discoideum Raper was selected for this study because of the unique advantages for experimental studies which it affords.

LITERATURE REVIEW

Brefeld (*2*), Van Tieghem (*22*), Olive (*14*), and Harper (*6*) noted the presence of bacteria in their cultures of species of the Dictyosteliaceae but did not understand the role played by the bacteria in the nutrition of the myxamoebae, nor did they know the identity of the bacteria that accompanied their cultures. Brefeld (*2*) noted the ingestion of solid particles by the myxamoebae of *Dictyostelium mucoroides*. Van Tieghem (*22*) and Olive (*14*) observed the actual ingestion of bacteria by myxamoebae of the Dictyosteliaceae, but both investigators believed this phenomenon to be without significance and concluded that the myxamoebae fed upon nutrients in solution. Inasmuch as the emphasis in their studies was placed on the fruiting stage, which involves the final orientation and differentiation of myxamoebae into a fruiting structure, rather than on the vegetative stage, which involves the growth and multiplication of myxamoebae, this lack of appreciation of the role of bacteria is understandable.

Nadson (*12*) was the first to call attention to the association of a known species of bacteria, *Bacillus fluorescens liquefaciens*, with a species of the Dictyosteliaceae, *Dictyostelium mucoroides*. He considered that the two organisms were symbionts and that the bacteria favored the slime mold by creating an alkaline reaction in the culture medium. The myxamoebae, he believed, fed solely upon nutrients in solution.

In his extensive study of the physiology of *Dictyostelium mucoroides*, 3 years later, Potts (*17*) observed that the myxamoebae of *D. mucoroides* were dependent upon the presence of bacteria for their nutrition and growth, and further that colonies of bacteria were consumed by the slime mold. He did not observe the ingestion of bacteria by the myxamoebae, however, and concluded that the myxamoebae excreted an enzyme, which digested the bacterial cells outside the bodies of the myxamoebae, and subsequently incorporated in solution the products of their decomposition. Using maize extract agar, he was able to grow *D. mucoroides* in association with pure cultures of *Bacillus megatherium*, *B. subtilis*, and *B. fluorescens liquefaciens*, in addition to *Bacterium fimbriatum* Potts with which he originally isolated the slime mold. Potts found no indication of a symbiotic relationship between the slime mold and the associated bacteria.

Vuillemin (*24*) isolated and cultivated *Dictyostelium mucoroides* in association with *Bacillus fluorescens non-liquefaciens*, but could not grow the same slime mold in association with *B. pyocyaneus*. He (*24*) reported that the slime mold acted as a "bacteriophage" and that the myxamoebae of this species ingested bacterial cells and digested them in vacuoles within the bodies of the myxamoebae. In the same year, Pinoy (*15*) reported successfully cultivating *D. mucoroides* in association with, or, as he termed it, in "pure-mixed culture", with *Micro-*

bacillus prodigiosus and *B. coli communis* in addition to *B. fluorescens liquefaciens*, which had been associated with the slime mold in his original isolation. Four years later (*16*) he added five additional Gram-negative bacteria to the list with which *D. mucoroides* could grow, but reported that it could not grow with Gram-positive species or, as Vuillemin (*24*) had indicated before him, with *B. pyocyaneus*. Pinoy (*16*) made an intensive study of the ingestion and digestion of bacterial cells by the myxamoebae of *D. mucoroides*. He succeeded in extracting from large numbers of myxamoebae a preparation which (1) liquefied gelatine and (2) dissolved bacterial cells (*B. coli communis*) that had been killed by chloroform. To the active substance, or enzyme, contained in this extract, he gave the name "acrasidiastase", and noted that on the whole it was similar in its action to a substance, or enzyme, earlier isolated by Mouton (*11*) from a soil amoeba and termed by him "amibodiastase." Pinoy (*16*) considered the slime mold as living parasitically upon the colonies of the associated bacteria.

Skupienski (*20, 21*) likewise isolated *Dictyostelium mucoroides* in association with *Bacillus fluorescens liquefaciens* and cultivated the two organisms in pure-mixed culture. Like Vuillemin and Pinoy, he reported the ingestion and digestion of bacterial cells by the myxamoebae of *D. mucoroides*, but believed nonetheless that the two organisms were symbionts.

In 1935 the writer (*18*) described *Dictyostelium discoideum*, a new species characterized by (1) migration, (2) upright sorocarps, and (3) discoid base, pointing out that this species was accompanied by bacteria and that the myxamoebae reached their maximum development within the bacterial colonies, and, indeed, occurred outside the limits of such only as scattered myxamoebae. However, neither the relation between the myxamoebae and the bacteria nor the identity of the associated bacteria was then known. Since that time these points have been cleared up in large measure, and this work is reported and discussed herein.

HOST RANGE OF DICTYOSTELIUM DISCOIDEUM

DICTYOSTELIUM DISCOIDEUM IN ASSOCIATION WITH VIBRIO ALKALIGINES

Using the type strain of *Dictyostelium discoideum* (*18*), the writer isolated the bacteria associated with this slime mold and found that it was accompanied by a single species of bacteria, *Vibrio alkaligines* Lehm. and Neum.[4] When grown upon hay-infusion agar this bacterial culture produces raised, somewhat slimy colonies, which more or less retain their general character even when a rich growth of *Dictyostelium* occurs within them.[5]

In culturing *Dictyostelium discoideum* in association with *Vibrio alkaligines* upon hay-infusion agar, the usual method of transfer was to remove loopfuls of bacteria and *Dictyostelium* from well-established cultures and to streak these upon the surface of fresh agar plates.

[4] Identified by N. R. Smith.

[5] As will be seen from the discussions that follow, this was not the case with the majority of bacterial species that were subsequently studied in association with *D. discoideum*, for in the greater percentage of cases the bacterial colonies were completely consumed by the feeding myxamoebae. The fact that the myxamoebae of *D. discoideum* only partially consume the colonies of *Vibrio alkaligines* explains in large measure why the writer overlooked the true relation between the myxamoebae and the associated bacteria during his early studies with this slime mold.

When this method was employed, good growth of bacteria and of *Dictyostelium* was invariably obtained. However, when care was taken to transfer only the spores from single sorocarps, growth of the *Dictyostelium* was not uniformly obtained but occurred at some points of inoculation and not at others. Further, in these cultures it was observed that the two organisms always grew at the same points and that neither ever developed alone. The explanation of this phenomenon was not at first obvious, but later experiments proved that the slime mold was dependent upon bacteria for nutriment, a fact which was in agreement with results earlier obtained by Potts (*17*) and Pinoy (*16*) with *D. mucoroides*.

From the foregoing results it was obvious that bacteria-free spores had been inoculated at some places but not at others. In order to determine the character and position of the sorocarps bearing spores that were free of bacteria, inoculations were again made with spores only, and care was taken to inoculate some plates with spores taken from sorocarps that had been formed outside the limits of the bacterial colonies in which the pseudoplasmodia had developed, while other plates were inoculated with spores taken from sorocarps that had been formed within the limits of the bacterial colonies.[6] It was found that bacteria and *Dictyostelium* grew at all points of inoculation in plates that had been seeded with spores obtained from sorocarps located within the bacterial colonies, but occurred at only 1 of 10 points of inoculation in plates that had been seeded with spores obtained from sorocarps located outside the bacterial colonies. Subsequent experiments of this type demonstrated that if spores were taken only from sorocarps which had formed at a distance of 0.5 cm or more beyond any evidence of bacterial growth then the spores were in all cases free from bacteria.

Such bacteria-free spores germinated, but the emergent myxamoebae failed to grow in the absence of bacteria. However, it was only necessary to add bacteria to any point at which pure spores had been inoculated in order to secure an immediate growth of *Dictyostelium discoideum*.

The pseudoplasmodia were positively phototactic, and when cultures were placed in one-sided illumination the pseudoplasmodia migrated toward the light. Under favorable culture conditions a large percentage of the resulting sorocarps were formed at distances of 1.0 cm or more from bacterial colonies in which the pseudoplasmodia developed. In migrating this distance, the pseudoplasmodia divested themselves of all bacteria that were originally either contained inside or attached to the pseudoplasmodia at the time they left the bacterial colony. *Dictyostelium discoideum* is the only species of the Dictyosteliaceae that possesses a migrating pseudoplasmodium and is, therefore, the only species that regularly bears bacteria-free spores. The pure spores borne in isolated sorocarps that are formed from migrating pseudoplasmodia can be inoculated at will with a pure culture of any bacterium, and the growth and development of *D. discoideum* in association with that bacterium can be studied in what Pinoy, Skupienski, and other workers have called a "pure-mixed culture."

[6] In *D. discoideum* (*18*), after the myxamoebae comprising the pseudoplasmodia have crowded together into compact, cylindrical, tapering masses, these masses, or pseudoplasmodia, characteristically move outside the bacterial colony as units and continue to migrate for a greater or less distance before forming sorocarps At the same time, other pseudoplasmodia fail to migrate and hence form sorocarps within the limits of the bacterial colony.

PRELIMINARY STUDIES WITH CERTAIN SAPROPHYTIC BACTERIAL ASSOCIATES

Since members of the Dictyosteliaceae had been isolated almost exclusively with fluorescent bacteria by earlier investigators (*12, 15, 20, 24*) and had been successfully grown in association with these bacteria by Nadson (*12*), Potts (*17*), Vuillemin (*24*), Pinoy (*15, 16*), and Skupienski (*20, 21*), the writer early turned to a study of *Dictyostelium discoideum* in association with *Pseudomonas fluorescens* Migula.[7] Following this, the slime mold was cultivated in association with an additional species of Gram-negative bacteria, *Escherichia coli* (Migula) Castellani and Chalmers, and with two Gram-positive species, *Bacillus megatherium* De Bary and *B. subtilis* Cohn. With each of these common saprophytic species, *D. discoideum* grew somewhat better than in control cultures with *Vibrio alkaligines*. Thus *D. discoideum* grew better in association with other bacterial species than that with which the slime mold was originally associated.

In the above-mentioned cultures with common saprophytic bacteria and in those with *Vibrio alkaligines*, the mature sorocarps varied greatly in size but were always of essentially the same pattern, characterized by upright, straight, sinuous, or loosely spiral sorophores, which arose from circular and expanded or cone-shaped basal disks and bore at their apices grayish-white to pale lemon-yellow sori of slightly elliptical form. Under favorable conditions such as existed in these cultures, the differentiation of myxamoebae in the developing sorocarp into stalk cells and spores progressed at a proportional rate, so that, regardless of the size of the fructification, the ratio of sorophore (stalk) to sorus (spore mass) size remained relatively constant. This condition is well illustrated by the camera lucida drawings of mature sorocarps of normal pattern shown in figure 1, *A–D*. For a detailed account of sorocarp formation, the reader is referred to the original description of *Dictyostelium discoideum* (*18*).

The preliminary studies clearly demonstrated: (1) That *Dictyostelium discoideum* could grow, and grow well, in association with bacteria other than that with which the original culture was associated; (2) that *D. discoideum* could grow alike with Gram-negative and Gram positive bacteria; (3) that hay-infusion agar provided a satisfactory medium for cultivating *D. discoideum* in association with different bacteria; (4) that the sorocarps produced in cultures with different bacteria presented essentially the same pattern; and (5) that the ratio of sorophore to sorus size remained relatively constant regardless of the size of the sorocarp. Therefore, it was recognized that *D. discoideum* afforded a particularly favorable species with which to study quantitatively the growth of a species of the Dictyosteliaceae in association with a large number of different bacteria belonging to diverse groups.

METHODS DEVELOPED FOR MAKING GROWTH COMPARISONS

For these comparative studies of the growth of *Dictyostelium discoideum* in association with various bacteria, hay-infusion agar was used, the composition [8] being kept as constant as possible.

[7] In discussing the writer's work, the nomenclature for the bacterial species studied is in all cases according to Bergey (*1*); whereas, in reviewing the work of earlier investigators, the nomenclature used by those investigators is followed.

[8] The hay-infusion agar was prepared in the following manner: 35 g of partially decomposed hay infused in a liter of tap water for one-half hour in an autoclave at 110° C. and 5–10 pounds pressure; infusion filtered, and filtrate made up to 1 liter; 0.2 percent K_2HPO_4 and 1.5 percent agar added; adjusted to ρH of 6.0–6.2; and sterilized at 15 pounds pressure for 20 minutes.

Triplicate plates of hay-infusion agar (each plate containing approximately 20 cc of medium) were inoculated with each of the cultures listed in tables 1 and 2. Inoculations were made in the following manner: Six giant colonies were established at regular intervals in a circle near the periphery of the plate, and a single colony was planted in the center; the inoculations were made with a small loop, and for each colony the inoculum was spread over an area of approximately 1 cm^2. In all cases the cultures were allowed to grow at room temperature of 22–24° C. for 2 to 3 days prior to the introduction of pure spores of *Dictyostelium*. The spores used as inoculum were obtained from sorocarps that had developed 0.5 cm or more beyond any evidence of bacterial growth. As an added precaution, spores from only one sorocarp were introduced into each bacterial colony, so that if contaminating bacteria were in any case introduced with the inoculum they would not be added to more than a single colony. Spores were

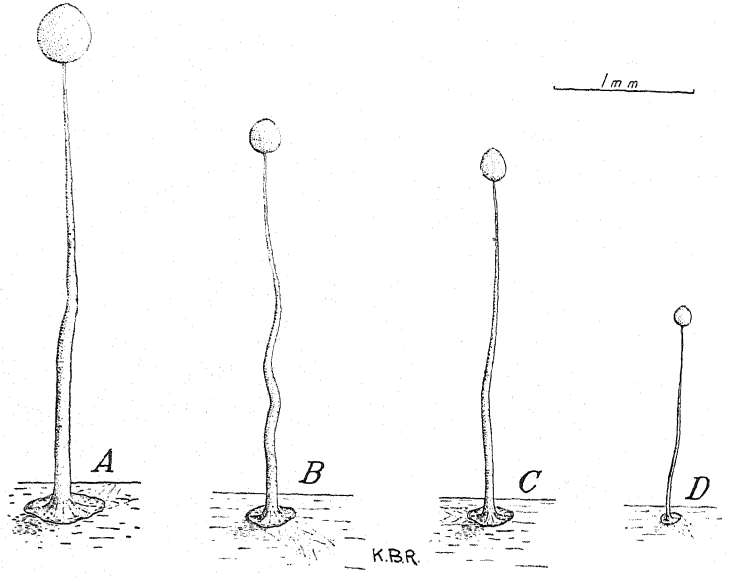


FIGURE 1.—Normal sorocarps of *Dictyostelium discoideum*: *A*, sorocarp of large size; *B* and *C*, sorocarps of medium size; *D*, sorocarp of small size. Whatever the size, the parts of the sorocarp—discoid base, tapering stalk or sorophore, and ellipsoid spore mass or sorus—are easily recognized.

introduced into each of the colonies in two of the three plates and into six of the seven colonies in the third plate. Thus for each bacterial species studied, *D. discoideum* was introduced into 20 colonies, while a single colony was reserved as a measure of the growth of the bacteria alone.

Incubation of the cultures was continued at room temperature after the spores were introduced. The cultures were observed from time to time, and a record was made of the time at which the first pseudoplasmodia began to form; and a record of the complete growth of *Dictyostelium discoideum* was made after sorocarp formation had ceased, which in the majority of cases was 6 to 8 days after the spores were added.

The total growth of *Dictyostelium discoideum* was determined by counting the number of large, medium, and small sorocarps in each of the 20 colonies of the several bacterial cultures. These counts were subsequently averaged and the growth per colony was obtained (tables 1 and 2).

For the terms "large", "medium", and "small sorocarps", used throughout this paper as a means of expressing quantitatively the growth of *Dictyostelium discoideum*, the following arbitrary limits were chosen:

(1) *Large sorocarps.*—Those with sori having a cross diameter of 250μ or more and sorophores having a length of 2.5 to 3.0 mm or more.
(2) *Medium sorocarps.*—Those with sori having a cross diameter of 150μ to 250μ and sorophores having a length of 2 to 3 mm.
(3) *Small sorocarps.*—Those with sori having a cross diameter of 75μ to 150μ and sorophores having a length of 1 to 2 mm.

Although it might seem that the number and size of the sorocarps would not serve as a reliable index of the amount of growth in *Dictyostelium discoideum*, an analysis of growth in this organism shows ample justification for such a procedure. Growth in the Dictyosteliaceae consists in an increase in the mass and in the numbers of feeding myxamoebae, that is, it is characteristic of the vegetative stage. On the other hand, the formation of the fruiting structures involves no vegetative growth but rather is a matter of development, or "integration and differentiation" as Harper (*8*) has termed it. Since this is true, it would at first seem that any comparison of growth should be limited to the vegetative stage where growth occurs. However, since no increase in the number of myxamoebae occurs after the fruiting stage is initiated and since each myxamoeba entering into a psuedoplasmodium forms either a spore or a stalk cell in the developing fructification (*2*, *3*, *6*, *14*), the identity of each vegetative myxamoeba is in fact preserved in the mature sorocarp. The vacuolate stalk cells are much larger than the spores, but since the size of the sorus and that of the sorophore remain proportional (p. 293), the relative numbers of the two cell types remain approximately constant in normal sorocarps. A sorocarp of a particular size, therefore, represents a fairly definite amount of actual growth. Since this is true, the amount of growth occurring in any bacterial colony can be expressed in the numbers of sorocarps produced in that colony, when the size of the sorocarps is considered.

The total growth of *Dictyostelium discoideum* in association with a given culture of bacteria can be satisfactorily expressed in terms of the total number of large, medium, and small sorocarps; but the growth of the slime mold with two or more different bacteria cannot be quantitatively compared in terms of the total number of sorocarps, because the numbers of sorocarps falling within the different size groups do not remain in the same ratio to one another.[9] However, such a quantitative comparison can be made and the results graphically shown, if the sorocarps of the three sizes are all converted into a common expression. As already noted, the amount of actual growth represented by a sorocarp is in direct proportion to the size of the

[9] This point is excellently illustrated by the growth and development of *Dictyostelium discoideum* with *Bacillus cereus* Frankland and Frankland and with *Sarcina lutea* Schroeter as shown in table 1. There was an average of 23 sorocarps per colony of *B. cereus* and an average of 22 sorocarps per colony of *S. lutea*; still the growth of the slime mold was almost three times as great with the latter associate (fig. 2). This apparent contradiction is explained entirely by the size distribution of the sorocarps in the colonies of the two bacteria (table 1).

sorocarp; and further, the relative proportions of sorus and sorophore remain fairly constant in a normal sorocarp regardless of the size of that sorocarp. Therefore the amount of growth represented by a sorocarp of one size can be expressed in terms of a sorocarp, or sorocarp equivalent, of another size simply by computing the volumes of their sori.

A basis for converting sorocarps of different sizes into a common expression was obtained in the following manner: (1) The average dimensions of 50 large, 50 medium, and 50 small sori were determined; (2) then, treating the sori as spheres,[10] the average volume of a large, medium, and small sorus was computed. Medium sori were chosen as the most convenient size to which to convert sorus volumes, and a medium sorus was found to be equivalent to 6 small sori and to 0.29 of a large sorus. The total amount of growth of *Dictyostelium discoideum* with the different bacteria is, therefore, expressed as medium-sorocarp equivalents wherever quantitative comparisons are made, as in figures 2 and 3.

Because of the amount of work involved, these investigations extended over a period of 6 months. However, the results obtained with the several species of bacteria could be quantitatively compared and the differences in the growth of *Dictyostelium* could be attributed either to the amount of bacterial growth present or to the ability of the myxamoebae to feed upon the bacteria, since the following factors remained constant throughout the study: (1) The culture media were approximately uniform in composition; (2) the number of bacterial colonies per plate and the area inoculated per colony were the same in all cases; (3) the cultures were all incubated at the same temperature, 22°–24° C; and (4) the bacterial colonies were all seeded with pure spores of *D. discoideum* when 2 to 3 days old.

DICTYOSTELIUM DISCOIDEUM IN ASSOCIATION WITH COMMON SAPROPHYTIC BACTERIA

A comprehensive study of the growth and development of *Dictyostelium discoideum* in association with a large number of different species of saprophytic bacteria was undertaken, and for this investigation species belonging to the following genera and families were selected: *Vibrio*, of the Spirillaceae; *Pseudomonas*, *Serratia*, *Escherichia*, *Aerobacter*, *Achromobacter*, *Cellulomonas*, and *Chromobacterium*, of the Gram-negative Bacteriaceae; *Bacillus*, of the Gram-positive, spore-forming Bacillaceae; *Rhizobium* and *Azotobacter*, of the nitrogen-fixing Nitrobacteriaceae; and *Sarcina* and *Micrococcus*, of the Coccaceae. A complete list of the species studied is given in table 1.

Inoculations with bacteria and subsequently with pure spores of *Dictyostelium discoideum* were made in the manner described above.

The cultures were observed from time to time and a record was made of the time at which pseudoplasmodium formation began, of the character of the sorocarps produced, and of the total growth of *Dictyostelium discoideum* in association with the several bacterial cultures after sorocarp formation ceased.

[10] As shown in fig. 1, *A*–*D*, the sori are not true spheres but are slightly teardrop or ellipsoid in shape; however, the percentage of error in computing them as spheres is small and is proportional for the different size groups.

TABLE 1.—*Comparative growth of Dictyostelium discoideum in association with different saprophytic bacteria on hay-infusion agar*

Bacterial associate			Growth of *Dictyostelium discoideum*								
Culture no.	Species according to Bergey (*1*)	Reaction to Gram stain	Extent of colony clearance	Period to beginning of pseudoplasmodium formation	Average number of sorocarps per colony			Average number of medium sorocarp equivalents per colony	Character of sorocarps		
					Large	Medium	Small		Sorophore	Basal disk	Sorus
				Days							
NC-4.1	*Vibrio alkaligines*[1]	−	Partial	3	7	27	12	53	Straight	Circular, flattened	White to pale yellow.
112	*Pseudomonas fluorescens*	−	Complete	1½–2	10	25	10	62	Straight or sinuous	Circular, expanded	Do.
75	*Pseudomonas schuylkilliensis*	−	do	2	9	22	7	55	do	Cone-shaped	Do.
76	do	−	do	2	10	16	8	52	do	Circular, flattened	Do.
110	*Pseudomonas aeruginosa*	−	Partial	3-3½	1	8	4	12	Straight	do	Do.
175	*Serratia marcescens*	−	Complete	1½–2	6	21	9	44	Straight, red in color	Cone-shaped, red	Pink to light red.
107	*Escherichia coli*	−	do	2	11	26	7	66	Straight or sinuous	Circular, expanded	White to pale yellow.
Pa	*Aerobacter aerogenes*	−	do	2½	11	18	9	58	Straight	do	Do.
P	*Chromobacterium violaceum*	−	Partial	4–5	4	13	3	28	Straight or weak spiral.	Circular, flattened	Do.
36	*Achromobacter radiobacter*	−	Complete	2	9	17	7	50	Straight or spiral	do	Do.
188	do	−	do	2	12	26	8	69	do	do	Do.
168	*Achromobacter globiforme*	−	do	2	9	22	14	56	Straight or sinuous	do	Do.
124	*Cellulomonas cellasea*	−	do	2-2½	6	17	20	41	Sinuous or spiral	do	Do.
135	*Cellulomonas liquata*	−	Partial	5		3	8	4	Straight and short	Cone-shaped	Do.
133	*Cellulomonas fima*	−	do	5		5	7	6	Straight	Circular, flattened	Do.
104	*Bacillus subtilis*	+	Complete	2-2½	10	25	11	62	do	do	Do.
161	*Bacillus megatherium*	+	do	2-2½	10	24	7	60	do	do	Do.
162	*Bacillus mesentericus*	+	do		5	11	8	30	do	Circular, compact	Do.
50	*Bacillus danicus*	+	do	2-2½	6	13	23	38	do	do	Do.
160	*Bacillus cereus*	+	Partial	3	1	9	13	15	do	do	Do.
165	*Bacillus mycoides*	+	Slight	3	1	6	8	11	do	do	Do.
450	*Rhizobium meliloti* (alfalfa)		Complete	2-2½	10	15	7	51	Straight or sinuous	Circular, flattened	Do.
240	*Rhizobium japonicum* (soybean)		Partial	2-2½	5	10	6	28	Straight or spiral	Circular, compact	Do.
11	*Azotobacter chroococcum*		do	2-2½	6	11	4	33	Sinuous or spiral	do	Yellow.
16	*Azotobacter vinelandii*		do	3	2	9	10	18	Straight	Cone-shaped	Pale yellow.
K	*Sarcina lutea*	+	Complete	2-2½	9	9	4	41	Straight or sinuous	Circular, expanded	Yellow.
43	*Sarcina flava*	+	do	5	12	26	7	69	Sinuous or straight	Circular, flattened	Pale yellow.
101	*Micrococcus* sp. (yellow)	+	do	3	8	15	4	44	Spiral	Circular, expanded	Do.

[1] Not listed in Bergey's Manual (*1*) either as a valid species or as a synonym.

The results of these studies are summarized and presented in table 1, the total growth of *Dictyostelium* being recorded in the average number of large, medium, and small sorocarps per colony and also in the average number of medium-sorocarp equivalents. A comparison of the amount of growth of *Dictyostelium* in association with the different bacteria is graphically presented in figure 2, where the average total growth per colony is expressed in medium-sorocarp equivalents.

These studies can best be considered by discussing the growth and development of *Dictyostelium discoideum* in association with particular species or groups of species of bacteria.

Dictyostelium discoideum grew well and developed normally in association with the following species of Gram-negative non-spore-forming bacteria: *Pseudomonas fluorescens*, two strains of *Ps. schuylkilliensis* Chester, *Escherichia coli*, *Aerobacter aerogenes* (Kruse) Beijerinck, *Achromobacter globiforme* (Conn) Bergey et al., and *Achromobacter radiobacter* (Beijerinck and Van Delden) Bergey et al. In all of these cases the bacterial colonies were completely consumed by the feeding myxamoebae, pseudoplasmodia began to develop within 1½ to 2½ days after the introduction of *Dictyostelium* spores, and the slime mold had virtually ceased growing by the sixth or seventh day after its inoculation. The sorocarps were entirely normal in pattern, with straight, sinuous, or loosely spiral sorophores; white to pale lemon-yellow sori; and circular and flattened or cone-shaped basal disks (fig. 1, *A–D*). In association with each of these bacterial species, the growth of *D. discoideum* was in most cases as abundant as with *Vibrio alkaligines*, the species associated with the original culture of the slime mold, and in some cases even more abundant (table 1; fig. 2).

The character of the growth of *Dictyostelium discoideum* in association with *Serratia marcescens* Bizio (*Bacillus prodigiosus* Flügge) was especially interesting. Upon hay-infusion agar this bacterial species produces a dilute-crimson pigmentation. The pigment is contained within the bacterial cells and is not water-soluble. The myxamoebae of *D. discoideum* ingest and digest the cell bodies of *S. marcescens*, but apparently cannot digest the pigment, which, as a result, collects in scattered vacuoles of varying size within the bodies of the feeding myxamoebae. These vacuoles of pigment are not excreted when the myxamoebae enter the fruiting stage, and consequently the pseudoplasmodia are distinctly red in color. This coloration persists throughout the subsequent migration of the pseudoplasmodia and even through the formation of the fruiting structures, so that the mature sorocarps are characteristically colored, varying in hue from pink to dilute red. In such sorocarps as these the spores themselves are uncolored, but the slime surrounding them contains red granules which lend a red coloration to the sorus as a whole. The coloration of the sorophore and basal disk consists of minute reddish particles which are applied to the inner surface of the walls of the cells comprising these structures. Pinoy (*16*) obtained a somewhat similar coloration in *D. mucoroides* by cultivating it with *B. kieli* (*S. marcescens*?).

After it was found that *Dictyostelium discoideum* produced red pseudoplasmodia and sorocarps when grown in association with

Serratia marcescens, a culture of *Chromobacterium violaceum* (Schroeter) Bergonzini (*Bacillus violaceus* Schroeter) was procured in the hope that fruiting structures of blue or violet coloration could be obtained in cultures with this bacterium. This objective, however, was not realized, for, although a fair growth of *C. violaceum* occurred on hay-infusion agar, no pigment was produced and the sorocarps that subsequently developed were colorless.

Colonies of *Pseudomonas aeruginosa* (Schroeter) Migula were partially consumed by the feeding myxamoebae, and the resulting sorocarps were of normal pattern. Both Vuillemin (*24*) and Pinoy (*16*) attempted unsuccessfully to cultivate *Dictyostelium mucoroides* in pure-mixed culture with this bacterial species. The difference between the results obtained by these investigators and those obtained by the writer can probably be attributed to the different media employed.

Fair growth and normal development of *Dictyostelium discoideum* occurred in colonies of *Cellulomonas cellasea* (Kellerman et al.) Bergey et al., and the bacterial colonies were wholly consumed by the feeding myxamoebae. Poor growth of *D. discoideum* occurred in association with *C. liquata* (McBeth and Scales) Bergey et al. and *C. fima* (McBeth and Scales) Bergey et al., but these cultures were particularly interesting from another standpoint. After the sorocarps matured, the *Cellulomonas* attacked the cellulose walls of the cells of the sorophores

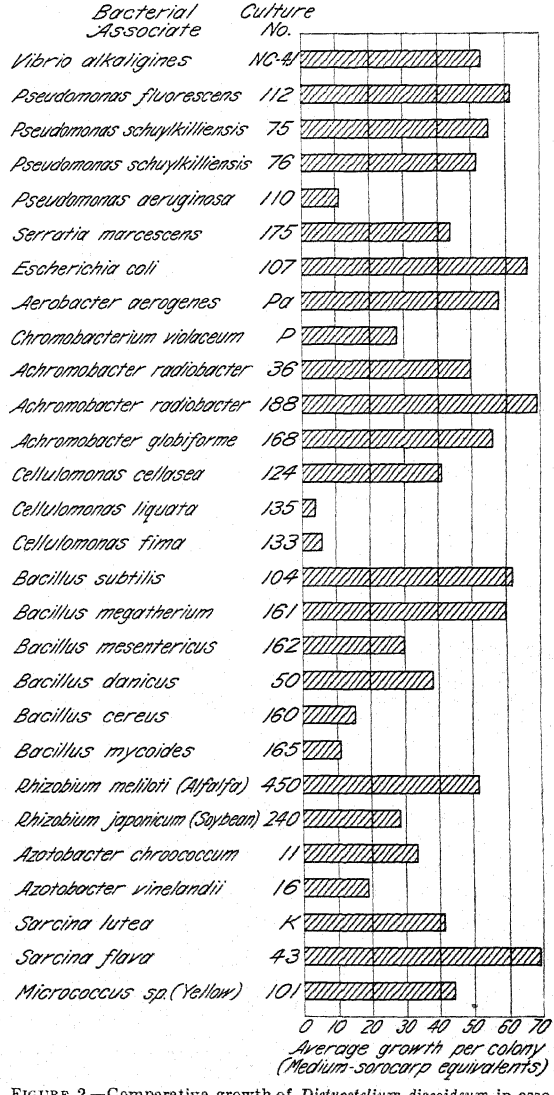


FIGURE 2.—Comparative growth of *Dictyostelium discoideum* in association with a number of common saprophytic bacteria growing upon hay-infusion agar.

and basal disks of the slime mold fructifications. In some cases all traces of cellular structure were obliterated, and the identity of the sorophore remained only as a very thin-walled hyaline tube, which contained rounded vacuoles of varying size. It may be noted that the cultures of *D. discoideum* in association with *C. fima* represent the nearest approach to a symbiotic relationship between slime mold and bacteria yet encountered among the Dictyosteliaceae, inasmuch as at first the myxamoebae fed upon the bacteria while later the bacteria decomposed the cellulose walls of the *Dictyostelium*. In these cultures, however, the growth of *D. discoideum* was so slight as to make a claim of true symbiosis untenable (table 1, fig. 2).

Among species of the Gram-positive, spore-forming bacilli, good growth of *Dictyostelium discoideum* occurred in association with *Bacillus megatherium* and *B. subtilis*, as in the preliminary studies discussed above; fair growth took place in association with *B. mesentericus* Trevisan and *B. danicus* Löhnis and Westermann; while only poor growth occurred in association with *B. cereus* and *B. mycoides* Flügge (table 1; fig. 2). The reduced growth of *Dictyostelium* in cultures with *B. mesentericus*, *B. danicus*, and *B. cereus* could largely be attributed to a smaller amount of bacterial growth and, therefore, of available food for the slime mold than was the case in cultures of *B. subtilis* and *B. megatherium*. On the other hand, *B. mycoides* grew well upon hay-infusion agar but the colonies were not noticeably cleared by the myxamoebae. The writer believes that the limited growth of the slime mold in this case can be attributed to the physical nature of the bacterial colonies. As is characteristic of *B. mycoides*, the bacilli were arranged in long chains that were not easily broken apart, and consequently the myxamoebae could not readily ingest the bacterial cells. There was no indication that the spores of any of the bacilli were digested, although in all cases they were ingested in large numbers by the myxamoebae. The sorocarps were of normal pattern in cultures with all bacilli studied.

Good growth of *Dictyostelium discoideum* occurred in association with *Rhizobium meliloti* Dangeard [11] (isolated from the nodules on the roots of alfalfa), and the bacterial colonies were completely consumed by the slime mold. On the other hand, only fair growth occurred in association with the related species *Rhizobium japonicum* (Kirchner) Buchanan (isolated from soybean), and the bacterial colonies were only partially cleared (table 1; fig. 2). No growth of *Dictyostelium* whatever occurred in colonies of *Rhizobium leguminosarum* Frank (isolated from vetch). An explanation of the lack of growth in this latter case has not yet been found; the bacteria grew well upon hay-infusion agar, and the physical character of the colonies was not essentially different from that of *R. japonicum*, in which a fair growth occurred. It is hoped that a further study of the growth of *D. discoideum* in association with different species and strains of bacteria belonging to this genus can be undertaken in the future.

Fair growth of *Dictyostelium discoideum* occurred in association with *Azotobacter chroococcum* Beijerinck, the bacterial colonies were partially cleared, and the sorocarps were of normal pattern; whereas poor

[11] The writer recognizes that members of the genus *Rhizobium* (*Bacillus radicicola* Beijerinck) are not saprophytic bacteria, but includes them here as a matter of convenience.

growth occurred in *Azotobacter vinelandii* Lipman, the bacterial colonies being only slightly cleared.

Dictyostelium discoideum grew well in association with *Sarcina lutea*, the bacterial colonies were wholly consumed, and normal sorocarps with yellow sori were produced. *D. discoideum* grew excellently (fig. 2) in association with *Sarcina flava* De Bary but developed more slowly than with *S. lutea* (table 1).

Moderately good growth of *Dictyostelium discoideum* occurred in association with a culture of a yellow *Micrococcus* (sp.?), and the bacterial colonies were completely consumed. The development of the slime mold in these cultures was especially interesting, for a large percentage of the mature sorocarps possessed closely spiral sorophores of regular and exquisite pattern.

DICTYOSTELIUM DISCOIDEUM IN ASSOCIATION WITH BACTERIA ISOLATED FROM CULTURES OF THE DICTYOSTELIACEAE

While studies with common saprophytic bacteria were being conducted, as described above, it was considered worth while to isolate the bacteria from other cultures of the Dictyosteliaceae which the writer had in culture and to attempt to grow *Dictyostelium discoideum* in association with them.

The bacteria associated with the several cultures of Dictyosteliaceae were isolated in pure culture by the dilution-plate method. The identity [12] of the bacterial cultures thus isolated, together with the name and catalog number of the culture of Dictyosteliaceae from which each was isolated, is given in table 2. From this table it will be seen that, of the writer's stock cultures of the Dictyosteliaceae, *Dictyostelium sphaerocephalum* was accompanied by three bacterial species and *Polysphondylium pallidum* (?) was accompanied by two species of bacteria, whereas all the others were accompanied by single bacterial associates. Conversely, three slime mold cultures were associated with *Vibrio alkaligines*, three with *Flavobacterium denitrificans* (Lehmann and Neumann) Bergey et al., two with *Pseudomonas schuylkilliensis*, two with *Aerobacter* or *Achromobacter* sp., and one each with *Ps. convexa* Chester, *Bacillus circulans* Jordan, and *F. buccalis* (Vignal) Bergey et al. There was no evidence pointing to an intimate relationship between the slime molds and the bacteria associated with them. It is interesting to note that *Ps. fluorescens*, which Nadson (*12*), Pinoy (*15, 16*), and Skupienski (*20, 21*) found habitually associated with *D. mucoroides* in Europe, did not occur in a single one of the writer's 10 cultures of Dictyosteliaceae from the United States (table 2).

Inoculation with bacteria and the introduction of pure spores of *Dictyostelium discoideum* into the resulting bacterial colonies were accomplished in the manner already described.

[12] Specific determinations made by N. R. Smith.

TABLE 2.—*Comparative growth of Dictyostelium discoideum in association with bacteria isolated from cultures of the Dictyosteliaceae*

Bacterial associate					Growth of *Dictyostelium discoideum*							
Species[1]	Reaction to Gram stain	Source		Colony character	Period to beginning of pseudoplasmodium formation (days)	Average number of sorocarps per colony			Average number of medium sorocarp-equivalents per colony	Character of sorocarps		
		Species of Dictyosteliaceae from which isolated	Culture no.			Large	Medium	Small		Sorophore	Basal disk	Sorus
Vibrio alkaligines	–	*Dictyostelium discoideum* Raper.	NC-4	Raised, smooth, moist	3	7	27	12	53	Straight	Circular, flattened.	White to pale lemon yellow.
Do	–	*Dictyostelium* sp.	D-8	Thin, smooth, moist	3–3½	4	30	11	46	do	do	Do.
Do	–	*Polysphondylium album* Olive or *P. pallidum* Olive.	P-8b	Raised, smooth, moist	3–3½	7	12	7	38	do	do	Do.
Pseudomonas schuylkilliensis.	–	do	P-8b	Slightly raised, smooth, glistening.	1½–2	15	29	13	83	Straight or spiral	Cone-shaped	Do.
Do	–	*D. sphaerocephalum* (Oud.) Sacc.	D-9	Thin, slightly granular, moist.	1½–2	9	20	12	53	do	Circular, flattened.	Do.
Pseudomonas convexa	–	*D. purpureum* Olive	D-6	Raised, smooth, moist	1½–2	6	16	7	38	do	Cone-shaped	Do.
Bacillus circulans	+	*D. mucoroides* Bref.	D-91	Thin, irregular, dryish	2	6	25	24	50	Straight	Circular, flattened.	Do.
Flavobacterium buccalis.	–	*D. sphaerocephalum*	D-9	Thin, dryish, spreading	2	5	10	1	28	Straight, simous, or spiral.	do	Do.
Flavobacterium denitrificans.[2]	–	*Dictyostelium* sp.	D-H	do	2–2½	12	21	12	68	do	do	Do.
Do	–	*D. sphaerocephalum*	D-9	Thin, smooth, spreading.	2–2½	8	12	13	42	do	do	Do.
Do	–	*P. violaceum* Bref.	P-1	Thin, dryish, spreading.	2–2½	8	22	20	53	do	Cone-shaped	Do.
Aerobacter or *Achromobacter* sp.	–	*D. mucoroides*	D-3a	Raised, smooth, moist	1½–2	8	22	6	51	do	Circular, flattened.	Do.
Do	–	do	D-12	do	1½–2	9	26	13	60	do	do	Do.

[1] Determinations made by N. R. Smith.
[2] A form belonging to the genus *Flavobacterium* and apparently like *F. denitrificans* except that nitrates are not reduced.

The results of these studies are summarized in table 2. The time at which pseudoplasmodium formation began is recorded; the character of the sorocarps is given; and the amount of growth of *Dictyostelium discoideum* in association with the several bacteria is recorded in the average number of medium-sorocarp equivalents as well as in the average number of large, medium, and small sorocarps per bacterial colony into which *Dictyostelium* was introduced. As in the preceding studies, the values given are averages obtained from detailed counts of 20 colonies for each bacterial culture. A graphic comparison of the growth of *D. discoideum* in association with the several bacterial cultures is given in figure 3.

As shown in table 2, *Dictyostelium discoideum* grew in association with each of the bacterial cultures isolated from members of the Dictyosteliaceae. The growth of the slime mold varied appreciably with the different cultures. Considering the study as a whole, approximately average growth of *D. discoideum* occurred in association with the strain of *Vibrio alkaligines*, No. NC–4.1, which was isolated from the type culture of this slime mold.

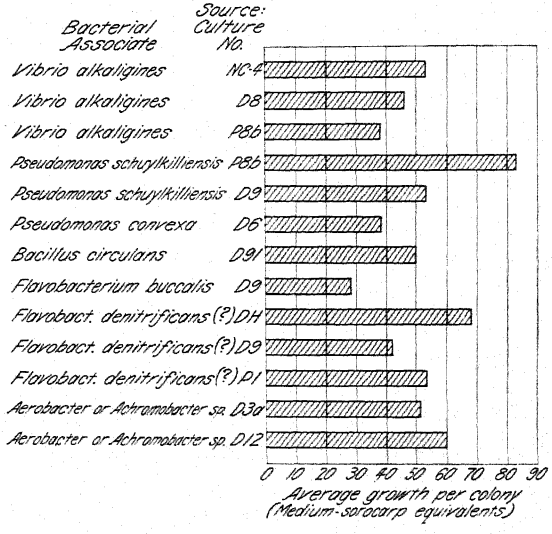


FIGURE 3.—Comparative growth of *Dictyostelium discoideum* in association with bacteria isolated from cultures of species of the Dictyosteliaceae. Cultures on hay-infusion agar.

The colonies of the three strains of *Vibrio alkaligines* studied were not wholly consumed by the *Dictyostelium*, whereas the colonies of all other bacteria isolated from members of the Dictyosteliaceae were completely devoured by the feeding myxamoebae, and the growth of the *Dictyostelium* was in direct proportion to the growth of the associated bacteria.

Pseudoplasmodia began to develop in 1½ days after spores of *Dictyostelium* were introduced into colonies of *Aerobacter* or *Achromobacter* sp. and species of *Pseudomonas*, whereas they did not appear in colonies of the three strains of *Vibrio alkaligines* until 3 days after spore inoculation. In colonies of *Bacillus circulans*, *Flavobacterium buccalis*, and *F. denitrificans*, the rate of development of the slime mold was intermediate between the above-mentioned extremes.

Normal sorocarps were produced in association with each culture of bacteria isolated from the Dictyosteliaceae. In all cases the sorocarps were characterized by evenly tapered, straight, sinuous, or loosely spiral sorophores; circular and flattened or cone-shaped basal disks; and white to pale lemon-yellow sori (table 2; fig. 1, A–D).

The growth and development of *Dictyostelium discoideum* in these cultures corresponded closely with that in cultures with common saprophytic bacteria, both with respect to the amount of growth present and with regard to the pattern of the sorocarps. There was no indication that bacteria which had been isolated from the Dictyosteliaceae were favorable for *D. discoideum* to a greater degree than common saprophytic bacteria, which, so far as known, had never been associated with a slime mold.

NUTRITION OF DICTYOSTELIUM DISCOIDEUM

FEEDING HABITS OF THE MYXAMOEBAE

Because of its large size, *Bacillus megatherium* was selected as a favorable form with which to study the manner in which myxamoebae of this group feed upon the bacteria that accompany them. Such an investigation seemed particularly pertinent because of the disparity in results obtained by Potts (*17*) and Pinoy (*16*) with the related species, *Dictyostelium mucoroides*.

Hanging-drop cultures were employed for this investigation, and hay infusion and hay-infusion agar were used as nutrient media. *Bacillus megatherium* and pure spores of *Dictyostelium discoideum* were inoculated together, and the growth of the slime mold was subsequently followed in microcultures by means of a Zeiss water-immersion lens. Corroborating the earlier work of Vuillemin (*24*), Pinoy (*16*), and Skupienski (*21*), it was found that the bacterial cells were ingested by the myxamoebae and digested in vacuoles within the amoeboid bodies.

The ingestion of bacterial cells and the subsequent progressive digestion of the same within the bodies of living myxamoebae could readily be followed. Different stages in the ingestion and digestion of cells of *Bacillus megatherium* are shown in figure 4. Figure 4, *A*, shows a myxamoeba shortly after its germination and before active feeding has begun; the hyaline ectoplasm (*ect*), the finely granular endoplasm (*end*), and the contractile vacuole (*cv*), are shown. The ingestion and digestion of bacilli by the myxamoebae of *Dictyostelium discoideum* is clearly shown in figure 4, *B*1–*B*7. The figures of this series show the same myxamoeba at approximately 10-minute intervals over a period of 65 minutes. In figure 4, *B*1, is shown a food vacuole (*v*1) of recent formation, and in the successive figures of the series the progressive digestion of the bacteria contained within it can be followed. Also shown in figure 4, *B*1, is the beginning of the ingestion of a chain of five bacilli. In figure 4, *B*2, the process of ingestion is further advanced, and in figure 4, *B*3, the first three cells of the chain have been cut off into a food vacuole (*v*2), and the digestion of the bacilli contained in it can be followed in the successive figures of the series. In figure 4, *B*4, the last two cells of the chain have been been completely ingested and are contained in a third food vacuole (*v*3) and the progressive digestion of its contents can likewise be followed.

From this series of figures a conception can be gained of the rapidity with which feeding, that is, the ingestion and digestion of bacteria, occurs; for in the course of approximately an hour, five bacilli were ingested and almost completely digested. The writer's studies indi-

cate that ingestion usually occurs at some point upon the anterior portion of the myxamoeba, but can occur and often does occur at any point upon the body surface. It should be remembered, however, that anterior and posterior regions in these amoeboid bodies mean little, since the identity of the two changes constantly.

During the digestion of *Bacillus megatherium* cells, certain changes in the form and content of the food vacuoles are regularly to be observed. These changes are shown diagrammatically in figure 4, *D*1 to *D*6. First, two bacilli are seen enclosed end to end in an elongate, ellipsoid vacuole. The vacuole tends to become spherical, and the two bacteria become separated and so displaced that they lie with their long axes either perpendicular or parallel to one another. During this time the vacuole becomes progressively smaller, and meanwhile an interesting change regularly takes place within the bodies of the

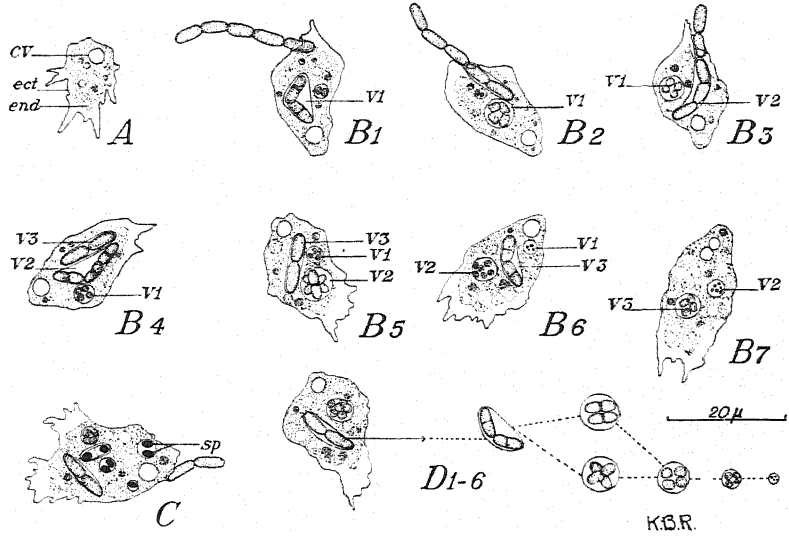


FIGURE 4.—Camera lucida drawings of myxamoebae of *Dictyostelium discoideum* feeding on cells of *Bacillus megatherium*. *A*, Young myxamoebae; *cv*, contractile vacuole; *end*, granular endoplasm; *ect*, hyaline ectoplasm. *B*1–*B*7, a feeding myxamoeba drawn at intervals of approximately 10 minutes over a period of 65 minutes; *v*1, *v*2, and *v*3, food vacuoles. *C*, Myxamoeba containing undigestible spores; *sp*, spores. *D*1–*D*6, food vacuole showing bacilli in progressive stages of digestion.

enclosed bacteria. Digestion first becomes evident near the middle region of an engulfed bacillus and becomes successively more distinct until the bacterial cell is completely digested at this point. The fractions of the original bacillus become more and more reduced in size and usually become further fragmented, while the vacuole becomes progressively smaller and finally disappears within the granular endoplasm of the myxamoeba.

Myxamoebae of *Dictyostelium discoideum* ingested and rapidly digested the vegetative cells of *Bacillus megatherium*, while the spores, although ingested in large numbers, were not digested (fig. 4, *C*).

The results of the writer's investigation upon the manner of feeding in *Dictyostelium discoideum* are in agreement with the studies reported by Vuillemin (*24*), Pinoy (*16*), and Skupienski (*21*) for *D. mucoroides*, while they were in marked disagreement with the results reported by

Potts (*17*), who reported that the bacteria were digested extracellularly by the myxamoebae of *D. mucoroides*.

In this connection it is interesting to note that in the Myxogastrales, a related order of the Mycetozoa, a somewhat similar ingestion and digestion of bacteria by the swarm cells occurs. This was first observed by Lister (*9*) and has since been studied in detail by Gilbert (*4*, *5*) and Smart.[13]

GROSS CLEARANCE OF BACTERIAL COLONIES BY THE MYXAMOEBAE

Working with *Dictyostelium mucoroides*, Potts (*17*) was the first to report the clearance and consumption of bacterial colonies by the myxamoebae of the Dictyosteliaceae, and this observation led to his discovery that *D. mucoroides* was dependent upon bacteria for its nutriment. In the case of *D. discoideum* the writer has found, as has been noted, that when spores of this slime mold are inoculated into colonies of bacteria upon hay-infusion agar the colonies are completely consumed in the majority of cases. Typically, the clearance of the colonies occurred in the following manner. After spores of *D. discoideum* are inoculated into the center of the bacterial colony, the first macroscopic evidence of the growth of the slime mold appears, approximately 18 to 24 hours later, as a small, cleared area at the point of inoculation. During the succeeding 18 to 24 hours, this area expands and the formerly smooth central area of the colony, now largely devoid of bacteria, becomes dull and minutely roughened. At the end of this period of approximately 1½ to 2 days the myxamoebae at the center of the colony begin the formation of pseudoplasmodia, and at the same time the cleared area is enlarged as the result of continued feeding by the remaining myxamoebae.

Among the factors that influence the rate and extent of clearance of the bacterial colony by the myxamoebae of *Dictyostelium discoideum*, may be listed (1) the character of the bacterial growth and (2) the composition of the underlying medium. It has already been noted that *D. discoideum* is able to feed only meagerly upon *Bacillus mycoides* because of the mycoid character of the bacterial growth and that the colonies of that species are not noticeably consumed. Also, *D. discoideum* grows poorly or not at all in bacterial colonies characterized by the presence of a large amount of gum. An investigation is now in progress on the influence of the composition of the medium on the rate and extent of colony clearance.

UTILIZATION OF KILLED BACTERIA BY THE MYXAMOEBAE

In the foregoing studies, *Dictyostelium discoideum* was in all cases grown in colonies of living bacteria, and the question arose as to whether living bacteria were essential to the growth of the myxamoebae and the subsequent development of a species of the Dictyosteliaceae. Some studies of a somewhat similar nature were made by earlier investigators. Potts (*17*) reported that *D. mucoroides* could grow upon cells of *Bacterium fimbriatum* that had been killed with chloroform but could not grow upon similar cells killed with alcohol or ether, and could not grow upon cells of *Bacillus megatherium* that had been killed by any agent. Pinoy (*16*) reported that *D. mucoroides*

[13] SMART, R. F. THE INFLUENCE OF EXTERNAL FACTORS ON THE BEHAVIOR AND DEVELOPMENT OF THE MYXOMYCETES. Unpublished Thesis, Ph. D., Harvard University. 1935.

could not grow upon cells of *Bacillus fluorescens liquefaciens* that had been killed by ether, chloroform, or other agents, and that the slime mold could grow only in the presence of living bacteria. The writer, therefore, undertook to determine whether *D. discoideum* could feed upon killed bacteria and, if so, whether normal development of the slime mold would subsequently take place.

Serratia marcescens and *Achromobacter radiobacter* were chosen for this study. Two experiments were performed, the bacteria in one being killed by heat and in the other by ultraviolet light. In the first of these experiments, colonies of the above-mentioned bacteria were allowed to develop for 3 days upon hay-infusion agar in Petri plates. At the end of that period the cultures, with the dish covers removed, were exposed to flowing steam in the autoclave for 10 minutes. The plates were then carefully removed in order that the floating bacterial colonies would not sink into the melted agar, and the medium was allowed to resolidify. To make certain the bacteria had been killed, nutrient agar tubes were inoculated with bacteria taken from the several colonies. When it was found that no viable bacteria remained, pure spores of *Dictyostelium discoideum* were introduced into the bacterial colonies. Good growth and normal development of *D. discoideum* occurred in the colonies of killed bacteria. The colonies were consumed in a manner similar to that already described for colonies of living bacteria.

In the second experiment, colonies of the above-mentioned bacteria were grown upon hay-infusion agar for 3 days and were then exposed to ultraviolet light.[14] As described above, nutrient agar tubes were inoculated with bacteria from the exposed colonies to make certain that the bacteria had been killed. Pure spores of *Dictyostelium discoideum* were then introduced into the exposed colonies and into similar colonies that had been shielded from the ultraviolet light. Better growth of *D. discoideum* occurred in the shielded (living) colonies than in the exposed (killed) colonies, but the development of the slime mold in the exposed colonies was entirely normal in character and sorocarps of normal pattern were produced (fig. 1, *A–D*).

UTILIZATION OF NUTRIENTS IN SOLUTION BY THE MYXAMOEBAE

Since the myxamoebae of *Dictyostelium discoideum*, contrary to what Pinoy (*16*) reported for *D. mucoroides*, were not obligately dependent upon living bacteria, the problem arose as to whether bacteria were really essential for the growth and development of species of *Dictyostelium*. Although limited time has prevented the writer from investigating this point exhaustively, some pertinent information has been obtained.

Bacteria-free spores of *Dictyostelium discoideum* were repeatedly inoculated upon various infusion media made from hay, dung, potatoes, carrots, peas, mushrooms, or yeast, and upon a number of synthetic media containing varying amounts of peptone and some carbohydrate. With two exceptions, no growth whatever of the *Dictyostelium* occurred in such cultures in the absence of bacteria, and in the two exceptions the growth of the slime mold was very slight. These exceptions occurred under the following circumstances: (1) Upon an agar medium made from the broth of canned green peas, a very limited vegetative

[14] The writer is indebted to Dr. William Arnold for making the light exposures.

growth of the myxamoebae occurred; the myxamoebae, however, remained widely scattered and no pseudoplasmodia whatever were produced. (2) A very limited growth of *D. discoideum* occurred in the absence of bacteria upon media containing 1 percent of lactose and from 3 to 5 percent of peptone; here again, myxamoebae were few in number and scattered; however, a few micropseudoplasmodia developed, and from these minute sorocarps were formed.

A third experiment was performed in order to determine whether the myxamoebae could feed upon products which were of bacterial origin but which were not contained within the bacterial cells. Large colonies of *Escherichia coli* were grown upon a favorable medium and when 3 days old were scraped off and suspended in approximately 10 volumes of water. This suspension was vigorously shaken and was then filtered through an L2 Chamberland filter. The unheated sterile filtrate was solidified with sterile agar, and Petri plates of this medium were subsequently inoculated with pure spores of *Dictyostelium discoideum*. Spore germination occurred, but no vegetative growth of the slime mold took place. Thus it was indicated that soluble products resulting from bacterial growth could not be used as nutriment by the myxamoebae of *D. discoideum*.

The writer's results obtained with peptone-rich agar are especially interesting, since Nadson (*12*) reported a meager growth of *Dictyostelium mucoroides* in the absence of bacteria in a solution containing phosphates and 5 percent of peptone. The correctness of Nadson's work was later questioned by Potts (*17*) and Pinoy (*16*), on the basis of their studies with that slime mold.

The studies reported by Nadson (*12*) on *Dictyostelium mucoroides* and the writer's studies on *D. discoideum* would indicate that through continued study some synthetic medium will probably be found upon which a fair growth and normal development of the Dictyosteliaceae can take place in the complete absence of bacteria.

RELATION BETWEEN DICTYOSTELIUM DISCOIDEUM AND ASSOCIATED BACTERIA

A role of importance was first attributed to the accompanying bacteria by Nadson (*12*), who, working with *Dictyostelium mucoroides* in association with *Bacillus fluorescens liquefaciens*, reported that the slime mold and associated bacteria were symbionts. He believed that the bacteria favored the *Dictyostelium* by creating an alkaline reaction in the culture, while the *Dictyostelium* favored the bacteria by supplying them with foodstuffs in the form of mucus, empty spore cases, etc. Skupienski (*21*) likewise considered *D. mucoroides* to be symbiotic with the associated bacteria. He based his belief upon the following points: (1) *D. mucoroides* was always associated with bacteria and the habitual associate was a single species, *B. fluorescens liquefaciens* (*12, 15, 20*); (2) bacteria were often seen clustered at the posterior end of the myxamoebae in the region of the contractile vacuole, and this he interpreted as indicating that the myxamoebae excreted something that favored the bacteria.

Potts (*17*) observed that *Dictyostelium mucoroides* grew only in the presence of bacteria and that it consumed the bacterial colonies in which it fed. He found no evidence to indicate that the bacteria

gained anything from the association, and concluded that since the slime mold could grow with at least four different species the relationship could not be one of symbiosis.

In terming *Dictyostelium mucoroides* a "bacteriophage", Vuillemin (*24*) obviously considered the relationship as one approaching parasitism of the bacteria by the slime mold. Going one step further, Pinoy (*16*) reported that the Dictyosteliaceae studied by him could grow only in the presence of living bacteria and considered that the myxamoebae were truly parasitic upon the bacterial colonies in which they grew.

If Ward's (*25*) definition, that symbiosis is the cooperation of two associated organisms to their mutual advantage, is accepted, the writer's investigations gave no indication of a true symbiosis between *Dictyostelium discoideum* and any of the bacteria that have at different times accompanied it. As has been shown, the myxamoebae of this species ingested and digested the associated bacteria, and the colonies of bacteria were in the majority of cases completely consumed. On the other hand, there was no evidence that the growth of the accompanying bacteria was enhanced in any way by the presence of the slime mold. Further, the fact that *D. discoideum* grew equally well with many species of bacteria points to an absence of symbiosis. If any degree of symbiosis had existed between the slime mold and the accompanying bacteria, the following conditions should have prevailed: (1) *D. discoideum* should have grown better in association with *Vibrio alkaligines*, the bacterium that accompanied the original culture, than in association with bacteria isolated from other species of *Dictyostelium;* (2) *D. discoideum* should have grown better in association with bacteria isolated from other species of *Dictyostelium* than in association with bacteria isolated from species of the related genus *Polysphondylium;* and (3) *D. discoideum* should have grown better in association with bacteria isolated from species of the Dictyosteliaceae in general than in association with the bacteria that, so far as known, had never been associated with these slime molds. As clearly shown by the experiments discussed above, such results were not obtained (tables 1 and 2).

On evidence from his own studies, the writer is led to consider the relationship between the two organisms as one of a modified or specialized type of parasitism of bacteria by the myxamoebae. The term "modified parasitism" is used because one does not usually think of an organism being parasitized by another organism of greater size that itself. On the other hand, if one considers a bacterial colony as a distinct entity in itself rather than as an assemblage of individuals (i. e., bacterial cells), then Pinoy's view that the myxamoebae are parasitic upon colonies of bacteria is thoroughly accurate. Perhaps more correctly the myxamoebae should be regarded as predatory on the bacteria.

It is often convenient to refer to the associated bacteria as "host" to the *Dictyostelium*, and to the different bacteria with which *D. discoideum* will grow as its "host range." It should, however, be remembered that these terms are not used here in the usual sense, for, as in the case of parasitism, one does not usually think of an organism being host to a much larger organism than itself.

DISCUSSION

Because of certain unique features in its developmental history, *Dictyostelium discoideum* is a peculiarly favorable species with which to study problems pertaining to the relation between a member of the Dictyosteliaceae and the bacteria that accompany it. The vegetative stage in this and other species of the genus is indistinguishable; and similarly, the formation of pseudoplasmodia in *D. discoideum* is like that in other Dictyostelia. After the formation of the pseudoplasmodia, however, marked differences in behavior occur. The compact pseudoplasmodium of *D. discoideum* typically leaves the bacterial colony in which it develops, migrates for a greater of less distance across the agar surface, and then builds an erect sorocarp. On the other hand, the pseudoplasmodia of other species of the Dictyosteliaceae do not migrate, but build their sorocarps directly from the points of origin of the pseudoplasmodia within the bacterial colony. From the standpoint of cultural studies this difference is of the greatest importance. In migrating across the agar surface, the pseudoplasmodium of *D. discoideum* divests itself of bacteria and subsequently forms an isolated sorocarp upon the sterile agar surface. Such sorocarps regularly bear spores that are free from bacteria. In other species, where the base of the stalk always remains within the bacterial colony, bacteria are carried up the sorocarp during its formation or ascend the sorocarp shortly after its development is complete, and the spores borne by it are not regularly free from bacteria.

Dictyostelium discoideum can at will be placed in pure-mixed culture with any pure culture of bacteria simply by inoculating spores from isolated sorocarps into colonies of the selected bacteria. The ease of this operation stands in sharp contrast with the methods employed by Potts (*17*) and Pinoy (*16*) for securing pure-mixed cultures of *D. mucoroides* in association with bacteria other than those with which they isolated the slime mold. Potts (*17*) initially isolated *D. mucoroides* in association with *Bacterium fimbriatum*. To secure a culture of the slime mold in association with *Bacillus megatherium* alone, he cultured *Dictyostelium* in successive colonies of *B. megatherium* until *Bact. fimbriatum* was wholly eliminated. The technique employed by Pinoy (*16*) was likewise tedious. *D. mucoroides* was grown in pure-mixed culture with *B. fluorescens liquefaciens*, and spores from such cultures were suspended in water and drawn into capillary tubes, which were subsequently exposed to a temperature of 56° C. for 2 minutes. This exposure, according to Pinoy's report, killed the bacteria, while the majority of the spores of *D. mucoroides* remained viable. Conversely, Skupienski (*20*) reports that he attempted to free spores of the same slime mold from *B. fluorescens liquefaciens* by this method and found that the bacteria were more heat-resistant than the *Dictyostelium* spores.

The present studies show that a member of the Dictyosteliaceae can grow in association with a much greater number and with a wider range of bacterial species than was known from previously published works. Nadson (*12*), Vuillemin (*24*), and Skupienski (*20*, *21*) cultivated *Dictyostelium mucoroides* in association only with the fluorescent bacteria with which they isolated the slime mold. Potts (*17*) succeeded in growing the same slime mold in association with three species of bacteria other than *Bacterium fimbriatum*, which originally

accompanied his culture of *Dictyostelium*. Pinoy (*15*, *16*) isolated *D. mucoroides* with *Bacillus fluorescens liquefaciens* and subsequently cultivated it in pure-mixed culture with seven additional species of bacteria. With the single exception of *Rhizobium leguminosarum*, the writer succeeded in growing *D. discoideum* in association with each of the 42 pure cultures, representing 32 species of bacteria, which were investigated in this survey. Included among this number were a large number of common saprophytic forms and several bacteria that had been isolated from cultures of Dictyosteliaceae. From the standpoint of classification and natural groupings, the species studied included members of the following families (after Bergey et al. (*1*)): Bacteriaceae, Bacillaceae, Coccaceae, Spirillaceae, and Nitrobacteriaceae (tables 1 and 2).

Particular attention was given in the present study to establishing standardized culture practices and methods that would enable one to study and compare quantitatively the growth of a species of the Dictyosteliaceae in association with different bacteria. Objections may possibly be raised to the methods employed. However, after extended study of the problem, the writer is convinced that so long as the culture medium is kept uniform in composition and in the amount used per plate and so long as the number of colonies and the disposition of these colonies on the culture plate are kept constant, the number (and size) of sorocarps produced per bacterial colony affords the most reliable and the most convenient measure of the growth of a species of the Dictyosteliaceae.

Appreciable variation in the amount of growth of *Dictyostelium discoideum* occurred in association with the different bacterial species, and even with different strains of the same species (figs. 2 and 3). This variation in growth of the slime mold could be attributed either to the amount of bacterial growth or to the availability of the bacteria. In the majority of cases, the bacterial colonies were wholly consumed and the amount of *Dictyostelium* growth was directly proportional to the amount of bacterial growth. In other cases, the conditions within the bacterial colonies were not entirely favorable to *Dictyostelium* and factors other than the quantity of bacteria governed the amount of growth of *D. discoideum*.

When grown upon hay-infusion agar, Gram-negative and Gram-positive bacteria alike afforded favorable nutriment for *Dictyostelium discoideum*. Thus the writer's investigations corroborate, in this regard, the results obtained earlier by Potts (*17*), who reported that *D. mucoroides* grew well with either Gram-negative (*Bacillus fluorescens liquefaciens* and *Bacterium fimbriatum*) or Gram-positive (*Bacillus subtilis* and *B. megatherium*) bacteria. Contrary to these results, Pinoy (*16*) reported that *D. mucoroides* could not grow with Gram-positive bacteria (*B. subtilis* and *B. megatherium*), and attributed Potts' (*17*) earlier success to impure cultures in which small Gram-negative bacteria accompanied unnoticed the larger Gram-positive bacilli. Vuillemin (*24*) and Pinoy (*16*) attempted unsuccessfully to grow *D. mucoroides* in pure-mixed culture with *B. pyocyaneus*, whereas the writer succeeded in cultivating *D. discoideum* in association with this bacterial species.

What, then, is the explanation of this lack of agreement among students of the Dictyosteliaceae? Can the disparity in results be explained by an essential difference between *Dictyostelium mucoroides*

and *D. discoideum* in their ability to feed upon certain bacteria? The writer's studies do not indicate such an explanation, for in his experience the growth of *D. mucoroides* and *D. discoideum* in association with the same bacterial species upon the same medium was comparable both in amount and in vigor. Furthermore, Potts and Pinoy obtained results which were diametrically opposed even when they studied the same species of slime mold (*D. mucoroides*) in association with the same species of bacteria (*Bacillus subtilis* and *B. megatherium*). The writer believes that the primary explanation of the difference in results obtained by students of this group consists neither in a difference between the species or strains of *Dictyostelium* studied nor in a difference between the strains of bacteria investigated, but in the difference between the media employed as substrata for the bacteria. Potts (*17*) used maize-extract media and noted that its chief advantage resided in the fact that its nutrients were relatively unavailable to the bacteria tested. The nutritive composition of the hay-infusion agar employed by the writer is not known, but it was not a rich medium and the nutritive materials it contained were not of a type that would be readily available to the bacteria studied. Upon the basis of the writer's studies with *D. discoideum* the following conclusion can be stated as a general rule The more dilute a medium is in nutrients, or the less accessible its nutrients are to a large number of different bacterial species, the more probable it is that a member of the Dictyosteliaceae will be able to grow in association with a large number of bacterial species upon that medium.

It is on this basis that the writer believes the difference in results obtained by students of the Dictyosteliaceae are to be explained. To understand the whole situation, the composition and the concentration of the media and the fermentive ability of the associated bacteria must be known. This matter is now being investigated.

Contrary to the views of Nadson (*12*) and Skupienski (*21*), who worked with *Dictyostelium mucoroides*, the writer found no indication of a symbiotic relationship between *D. discoideum* and the bacteria with which it was associated. Rather, the myxamoebae of the slime mold should be considered as predatory upon the accompanying bacteria. This view, in general, conforms to those earlier set forth by Potts (*17*), Vuillemin (*24*), and Pinoy (*16*). There was no indication that *D. discoideum* could grow better in association with bacteria which had been isolated from cultures of Dictyosteliaceae than in association with common saprophytic bacteria which, so far as known, had never been associated with slime molds. Furthermore, there was no indication that the Dictyosteliaceae are habitually associated in nature with the fluorescent bacteria as indicated by the investigations of earlier workers (*12, 15, 20, 24*). The evidence would indicate that it is purely a matter of chance what soil bacteria the slime mold happens to be growing with at the time isolation is made. It is not considered significant that the majority of the writer's cultures of the Dictyosteliaceae were associated with single species of bacteria (table 2), for other species of bacteria, which probably accompanied the slime molds at the time of their original isolation, could have been weeded out by the repeated transfers incident to laboratory cultivation.

Finally, the question arises concerning the possible significance of the fact that *Dictyostelium discoideum* is able to grow in association

with a large number of bacterial species belonging to widely separated groups. *D. discoideum* is not widely distributed in nature and it is probable that this slime mold is not an active constituent of the soil flora. On the other hand, from the work of Raper and Thom (*19*) it is known that other species of this group are common in soil and in decaying vegetation, and it is possible that these may play a more important role in soil economy than has hitherto been attributed to them. Assuming that such common forms as *D. mucoroides* and *Polysphondylium violaceum* can likewise feed upon an equally wide variety of different bacteria, it is entirely possible that under certain circumstances they may alter the bacteriological population of the soil appreciably.

Another significant point brought out by these studies consists in the fact that *Dictyostelium discoideum* is shown to be an exceptionally favorable organism with which to study experimentally the relation and interaction between a small amoeba (i. e., myxamoeba) of the soil and the bacteria upon which it feeds. The following characters recommend it for such experimental studies: (1) It is easily cultivated in the laboratory; (2) it will feed upon a wide range of bacteria belonging to diverse groups when these are grown upon favorable media; (3) it can be placed in pure-mixed culture with any bacterial species at will by selecting isolated sorocarps as spore sources; and (4) it forms characteristic fruit structures which immediately identify the species.

SUMMARY

Dictyostelium discoideum regularly grows within the limits of bacterial colonies, but the pseudoplasmodia typically leave the bacterial colonies in which they develop and migrate for a greater or less distance across the agar surface before forming sorocarps.

During their migration, the pseudoplasmodia divest themselves of all bacteria, and the spores borne in sorocarps formed at a distance of 0.5 cm or more beyond the limits of the bacterial colony are regularly free from bacteria.

The type culture of *D. discoideum* was accompanied by the single bacterial species *Vibrio alkaligines* Lehm. and Neum. However, by selecting isolated sorocarps, which are bacteria-free, as spore sources, the slime mold could be readily grown in pure-mixed culture with other bacteria.

Standardized culture practices were employed and methods were developed whereby the growth of *D. discoideum* in association with different bacteria could be quantitatively compared. The amount of growth was ascertained by determining the average number of large, medium, and small sorocarps per colony. Quantitative comparisons of the growth with different bacterial associates were made by converting the sorocarps of all sizes into terms of medium sorocarps. For such a unit the term "medium-sorocarp equivalent" has been used.

D. discoideum was grown in association with a large number of saprophytic bacteria, including representatives of such diverse groups as the Gram-negative, nonspore-forming Bacteriaceae, the Gram-positive, spore-forming Bacillaceae, the Coccaceae, the Nitrobacteriaceae, and the Spirillaceae.

In the majority of cases, the bacterial colonies were completely consumed by the feeding myxamoebae, and the amount of growth of *D. discoideum* was directly proportional to the growth of the bacteria. In other cases, the amount of growth was governed by the physical character of the bacterial colonies or by unknown factors.

D. discoideum grew well in association with either Gram-negative or Gram-positive bacteria. On the whole, somewhat better growth occurred with Gram-negative than with Gram-positive species. However, there were exceptions to this rule, for very poor growth of the slime mold occurred with some Gram-negative species, whereas very good growth occurred with some Gram-positive forms.

The growth and development of *D. discoideum* in association with bacteria isolated from other members of the Dictyosteliaceae closely resembled that with common saprophytic bacteria. There was no indication that the Dictyosteliaceae were regularly associated in nature with any particular species or group of bacteria.

The sorocarps were essentially alike in all cultures studied except those with *Serratia marcescens*, where the sorocarps were of normal form but were red in color owing to the presence of a bacterial pigment, and those with *Micrococcus* sp., where the sorocarps were of normal color but were characterized by closely spiral sorophores.

The myxamoebae of *D. discoideum* feed by the ingestion and digestion of bacterial cells. Spores of the Gram-positive bacilli are ingested but are not digested.

The myxamoebae of *D. discoideum* can feed upon bacterial cells that have been killed by heat or by exposure to ultraviolet light; a less lxuriant but normal growth and development of the slime mold occurs in such cultures.

A very meager and abnormal vegetative growth of *D. discoideum* was obtained upon pea-broth agar and upon peptone-rich agar, in the total absence of bacteria.

The myxamoebae of *D. discoideum* are predatory upon the accompanying bacteria and there is no indication that the bacteria gain anything from their association with the slime mold. Therefore, the relationship between the two organisms cannot be regarded as one of symbiosis.

D. discoideum affords a particularly favorable organism with which to study problems pertaining to the relation between a small amoeba of the soil and the bacteria upon which it feeds.

Species of the Dictyosteliaceae may be capable of appreciably altering the bacteriological flora of decaying vegetation in soils.

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SUSCEPTIBILITY TO CRONARTIUM RIBICOLA OF THE FOUR PRINCIPAL RIBES SPECIES FOUND WITHIN THE COMMERCIAL RANGE OF PINUS MONTICOLA[1]

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INTRODUCTION

White-pine blister rust (*Cronartium ribicola* Fisch.) was discovered in western North America in the fall of 1921 (*1*).[3] By that time it had become distributed in eastern North America over a large section of the range of northern white pine (*Pinus strobus* L.), where measures were being energetically applied for its control (*13, pp. 80–90*). Earlier reconnaissance for the disease in the western portion of the continent had given negative results, and as for some years rigid quarantines had been in effect to prevent its introduction into western territory, hope was felt that the West, with its magnificent stands of western white pine (*P. monticola* Dougl.) and sugar pine (*P. lambertiana* Dougl.) might be kept free from this destructive parasite (*13, p. 82*). Scouting in 1922, however, showed the disease to be widely distributed on ribes[4] in the coastal region west of the Cascade Range, from southwestern Washington to the northern end of Vancouver Island in British Columbia, and over a large area about 100 miles north of the international boundary in the interior of British Columbia in the region that included the towns of Canoe, Revelstoke, and Beaton. Within this range it was found on western white pine at numerous points from the Puget Sound section of Washington to the northern limits of this species about 150 miles north of the international boundary, and also at several points in the infected area in the interior of British Columbia (*10*).

Studies showed that the disease had been present on pines in some of the infected areas for several years, evidently having been introduced near Vancouver, British Columbia, about 1910, and that it was spreading and intensifying with great rapidity (*5, 10*). Evidence secured in 1922 and 1923 indicated that wind-borne aeciospores were annually infecting ribes at distances of over a hundred miles from the centers of pine infection and aeciospore production (*5, 10*). At this rate of spread it was obvious that the rust would soon invade the commercial range of western white pine, which was only a little over

[1] Received for publication Dec. 5, 1936; issued September 1937.

[2] The authors are under obligation to J. S. Boyce, Division of Forest Pathology, under whose general supervision the study was begun; to G. B. Posey, formerly of the Division of Plant Disease Control, Bureau of Entomology and Plant Quarantine, for assistance and advice given in developing the basic plan of study; and to W. V. Benedict, C. N. Partington, E. M. Hornibrook, L. D. Lloyd, J. W. Kimmey, C. J. Nusbaum; A. A. McCready, and T. S. Buchanan, who assisted in the collection of the data. Especial indebtedness is acknowledged to the late A. T. Davidson, in charge of blister rust investigations for the Dominion Government, to H. T. Güssow, Dominion botanist, and to other members of the Dominion and Provincial Governments for their friendly cooperation during the course of the investigations in Canada.

[3] Reference is made by number (italic) to Literature Cited, p. 345.

[4] The genus name *Ribes* and the common noun ribes are used in this paper to include both currants and gooseberries.

100 miles south of the infected area in the interior pine belt of British Columbia. This situation made urgent the development of measures for the protection of the pine area of northern Idaho and adjacent Montana and Washington, and control operations were promptly instituted. Spread since 1922 has borne out these assumptions, for the rust was first found there in 1928 and is now well established within the area (*11, 20, 21*).

One of the first problems in connection with research on the disease in the West was to determine the susceptibility of western host plants, both pines and ribes (*5, pp. 875–877, 881–882*). In both cases information was meager, consisting, in the case of the pines, principally of evidence of high susceptibility in *Pinus flexilis* (*13, p. 75*), and, for the various species of ribes, being based only on limited inoculation tests (usually in the greenhouse) in the eastern part of the United States (*13, pp. 18–20*).

Preliminary observations in western pine infection centers were alone sufficient to demonstrate that western white pine was highly susceptible, evidently more so than northern white pine,[5] and studies were started in 1922 to determine the character of infection on such ribes species as were present in the infected area. The primary object of the studies was to obtain information applicable to the development of measures for the control of the rust. In view of the impending spread of the disease into the commercial range of western white pine, the most urgent problem in the investigations was to obtain a knowledge of the susceptibility and telium-producing capacity of the rust on the important ribes species in that region. The present paper reports the results of these investigations.

RANGE LIMITS OF WESTERN WHITE PINES IN RELATION TO SPREAD OF THE RUST

White pine species of the region, including the infected areas and the Idaho white pine and California sugar pine stands, are western white pine, whitebark pine (*Pinus albicaulis* Engelm.), limber pine (*P. flexilis* James), bristlecone pine (*P. aristata* Engelm.), foxtail pine (*P. balfouriana* Murray), and sugar pine. These species are all known to be susceptible to the rust (*9, 13, 14*). Their composite botanical ranges in this region and the range of the fungus as determined in 1922 and in 1932 are shown in figure 1.

While some of these pine species are valuable only for aesthetic or watershed-protection purposes, the commercial value alone of western white pine and sugar pine must be calculated in hundreds of millions of dollars, and their ultimate value to the region in which they occur is much larger.

Western white pine occurs in two belts, which may be designated "coastal" and "interior." The coastal belt is one of scattered occurrence of the species. It extends from the southern portion of the Sierra Nevada in California, northward through western Oregon and Washington, mainly along the Cascade Range, to Puget Sound, and thence through the coastal region of northwestern Washington and southwestern British Columbia to about the northern end of Vancouver Island. The interior belt centers in the area of northern Idaho, northeastern Washington, and western Montana and stretches

[5] BOYCE, J. S. PATHOLOGICAL INVESTIGATIVE WORK. Third Western White Pine Blister Rust Conf., Portland, Oreg., 1922, Rept. Proc., pp. 56–60. [Mimeographed.]

northward into British Columbia for about 200 miles. The main commercial range of western white pine is confined to the States mentioned, where the lumber industry is largely dependent upon this tree for its existence.

Wild ribes are widespread over the entire territory, both inside and outside the range of the pines.

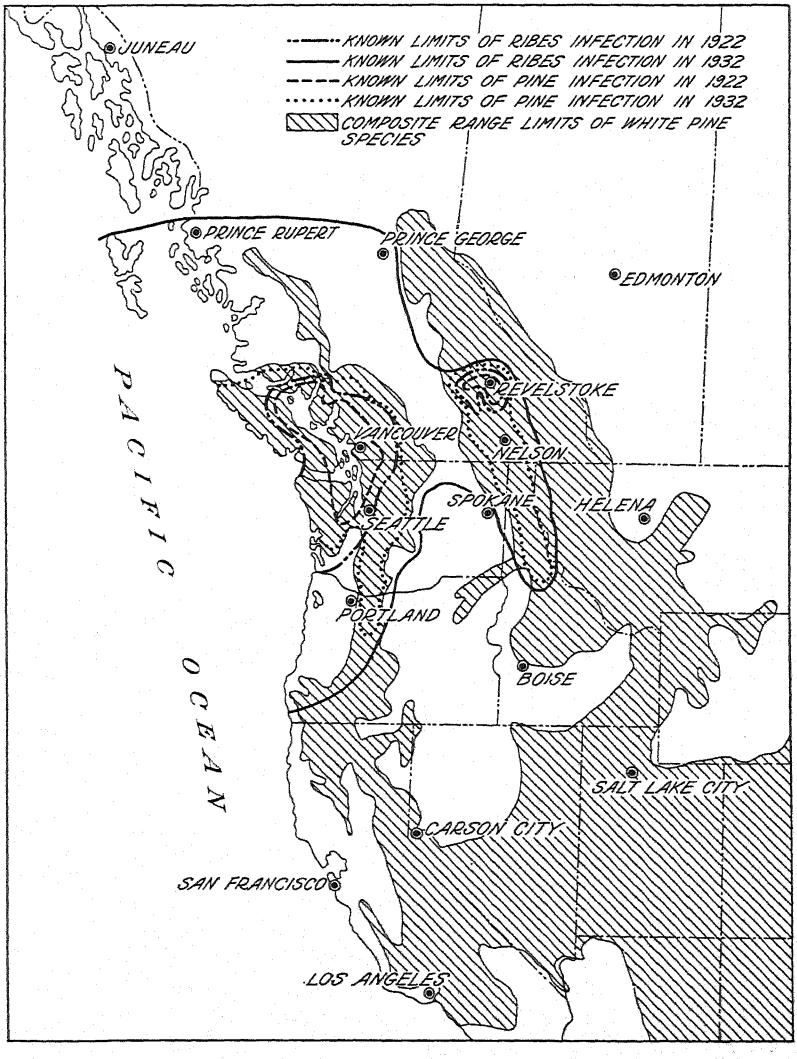


FIGURE 1.—Composite botanical range limits of white pine species in the West and known range of white-pine blister rust in 1922 and 1932.

From the distribution outlined above and from the map (fig. 1), it is evident that continuous belts of susceptible white pine exist through which the rust may be expected to spread from its known areas of distribution in 1922, over both the coastal and the interior

range of western white pine. The known range of the rust in 1932 indicates that the expected spread is in rapid progress.

RIBES SPECIES INVOLVED AND PREVIOUS STUDIES OF THEIR SUSCEPTIBILITY

At the time the rust was discovered in the West in 1921, little was known concerning the character or extent of association of ribes with commercial western white pine. By the end of 1923, however, reconnaissance [6] had indicated that for control purposes the commercial pine area centering in Idaho falls naturally into two main types—the stream type, which consists of moist stream-bank and bottom land characterized by profuse occurrence of ribes; and the timber type, which extends up and away from the stream type and in which ribes are more scattered. The most common species found in the stream type are *Ribes lacustre* (Pers.) Poir., *R. petiolare* Dougl., and *R. inerme* Rydb., the last two usually occurring in dense concentrations. The common species of the timber type are *R. viscosissimum* Pursh and *R. lacustre.* The last-named is generally more abundant numerically in both types than any of the other species. Species other than those mentioned are seldom encountered in either type.

When the study was started little was known of the susceptibility of three of the four ribes species mentioned, and nothing at all of the fourth, *R. petiolare.* Spaulding in 1922 (*13, pp. 18–20*) reported that a medium degree of infection developed on a relatively few bushes of *R. lacustre, R. viscosissimum,* and *R. inerme* when they were subjected to tests in the greenhouse and that slight infection developed on *R. lacustre* out of doors. Preliminary results of the present studies were reported in 1926 (*5*), showing susceptibility and telium-producing capacity to be high for *R. petiolare* and *R. inerme* and relatively low for *R. viscosissimum* and *R. lacustre.* In 1928, Hahn (*2, p. 680*) published results of greenhouse tests with these species in the East, which generally corroborated Spaulding's earlier results there and also showed *R. petiolare* to be susceptible. In neither Spaulding's nor Hahn's results, however, were any data given on the telium-producing capacity of the ribes, which is a primary consideration in control. Moreover, with the exception of *R. lacustre,* these results include nothing with regard to the reaction of the species out of doors.

Spaulding states, with respect to greenhouse inoculations (*13, p. 16*), "It was felt that greenhouse tests alone were not dependable for susceptibility data." The writers concur in this opinion. Hart (*4, pp. 930, 944–945*), working with stem rust of wheat, mentions instances in which tests conducted under the unnatural conditions of the greenhouse have given results contradictory to those secured in the field. Because of the wide variety of conditions found in nature, it was believed that only comprehensive studies out of doors under different site conditions could give a reliable index to the susceptibility of such variable hosts as ribes. These hosts have different growth forms within each species which differ as widely from one another in susceptibility as does one species from another. To reproduce these different forms and conditions on a sufficient scale in the greenhouse

[6] By the western branch of the former Office of Blister Rust Control, Bureau of Plant Industry, now comprised in the Division of Plant Disease Control, Bureau of Entomology and Plant Quarantine, U. S. Department of Agriculture.

would be quite out of the question. Accordingly, the studies reported in this paper were conducted out of doors.

METHODS

DEVELOPMENT OF STUDY METHODS

Determination of the general susceptibility under natural conditions of the four most common species of ribes in the Idaho-Montana-Washington area, has been one of the principal objectives of studies of the rust in the West. In the reaction of any species of ribes to *Cronartium ribicola*, two features are of outstanding importance in control: (1) The relative importance of a species and its various forms in the long-distance spread and local establishment of the rust in disease-free regions; (2) its ultimate capacity, after the rust has become established in a locality, for spreading infection to associated pines. The first logical step was to obtain a measure of the general susceptibility of the species, both to infection by aeciospores and intensification by urediospores, together with its capacity for producing telia from which the pine-infecting sporidia are developed.

After the inauguration of these studies, information became available (*15, p. 915*) which indicated that the relationship between teliospore production and the production of sporidia was not the same on all ribes species, since under natural conditions teliospores remained viable for much longer periods on some species than on others. Also, the possible existence of varying degrees of longevity and virulence in sporidia from different ribes hosts may be inferred from the results of studies reported at about the same time (*12, p. 589; 16, p. 419; 22, pp. 508–509*). The potential pine-damaging powers of various ribes, therefore, cannot be considered a direct function of their capacity for teliospore production, but until further experiments provide more definite information on this point an approximately constant relationship must be assumed.

Before any satisfactory tests of ribes susceptibility could be made, however, it was necessary first to devise a simple method of recording infection data that would permit the averaging of results and a ready comparison of the data of different years and places. The system used by Spaulding (*13, p. 17*) of differentiating the infection merely by the terms "light", "medium", and "heavy" was inadequate, because it did not permit the averaging of results and because the definitions of such terms vary considerably with the individual observer. Furthermore, the present study was concerned primarily with the telium-producing capacity of the ribes as a preliminary measure of their potentialities for transmitting the disease to the pines. Spaulding's system gave no measure of this capacity. Systems devised by investigators of other rusts (*7, 17, 19*) were equally inapplicable because of differences in the character of hosts and study objectives.

Concentrating on the features primarily essential to control, as given at the beginning of this section, and working with naturally infected plants in the vicinity of infected pines in 1923 and 1924, the following system of study was devised:

(A) By individual bushes:
  (1) Count of leaves produced.
  (2) Count of leaves infected.
  (3) Estimate of percentage of surface infected on infected leaves.
  (4) Count of rust-killed leaves dropped from bushes.

(5) Estimate of percentage of infected surface which—
  (*a*) Bore uredia.
  (*b*) Had borne uredia but had died before producing telia.
  (*c*) Had borne uredia but had fallen before producing telia.
  (*d*) Was necrotic, i. e., dying before producing any spore stages.
  (*e*) Bore telia.

(B) By groups of bushes:
  (1) Counts and estimates of leaves by individual bushes.
  (2) Count of bushes infected.
  (3) Estimate of percentage of leaves infected on infected bushes as a group.
  (4) Same as under 3, system A, for the group as a whole.
  (5) Estimate of percentage of rust-killed leaves dropped for the group as a whole.
  (6) Same as under 5, system A, for the group as a whole.

In addition to the observations outlined above, descriptive notes were taken on any abnormalities encountered and on the general character of infection and sporulation. Representative specimens of the infection were collected for each species and form to serve as permanent records supplementary to the data taken at each examination.

These systems were found to permit rapid collection of data and to give readily comparable results of sufficient accuracy for practical purposes. In general, after a little experience, percentage estimates of the different recorders were numerically within 1 to 5 or less of one another in the lower and upper percentage levels, and within less than 10 of one another at percentages around 50. Errors were usually compensating. System A was used where comparison between individual bushes was necessary. Where comparison between groups was the primary consideration, system B was used with such modifications or additions as were desired for individual comparisons. This latter system was far more rapid than the former in covering large numbers of bushes, and, although somewhat less accurate as far as any one group of plants was concerned, it decreased the probable error in the final results by permitting the use of a much larger basis.

Collection of the data by the systems just described made it possible to compute these data by simple arithmetical methods. In this connection the two things of primary consideration in the study were (1) percentage of total leaf surface infected, which was obtained by multiplying the percentage of the total number of leaves infected on a group of plants by the average percentage of surface infected on the infected leaves, and (2) the percentage of total leaf surface bearing telia, obtained by multiplying the percentage of total leaf surface infected by the percentage of infected surface bearing telia.

SELECTION OF STUDY AREAS

Prior to the inauguration of this study the wide spread of the rust by wind-borne aeciospores was practically confined to the extremely susceptible cultivated black currant (*Ribes nigrum* L.). The disease was very scarce and seldom found on other ribes species outside the general vicinity of infected pines. Of the four ribes species in question, *R. lacustre* was at that time the only one found close to infected pines in sufficient numbers to give any definite information of its susceptibility. Plants of the four species were therefore introduced and planted near infected pines in the spring of 1923. These plantings were made mainly with cuttings and gave but poor survival and results.

During the season of 1923 the rust was widely distributed by the wind to cultivated black currant plantings over the dry belt of British Columbia, lying between the coastal and interior white pine belts. This belt contains an abundance of all four of the ribes species studied, which are well adapted to this region and grow prolifically. Since pines were absent from the area, since spread of the rust from ribes to ribes is slight as compared with the distance of spread from pines to ribes, and since it was evident that the rust did not overwinter on ribes in the region, this belt offered a splendid opportunity to make inoculation tests of naturally growing plants of these species under comparable conditions without any danger of permanently establishing the disease or hastening its spread. Tests were accordingly begun there in 1924 near Kelowna, British Columbia, where the rust had been abundant on cultivated black currants in 1923. To this study locality were added two others in the same belt in 1926, one each near Summerland and Oliver at elevations similar to Kelowna (table 1), and

TABLE 1.—*Description of study areas*

Area	Elevation	Soil[1]	Slope and exposure	Vegetation[2]	Remarks
Interior British Columbia:					
Near Kelowna:	*Feet*				
Swamp area	1,150	P, t	Flat	G, S, A, B	Wet, poorly drained flat. Somewhat more rain than at Kelowna.
Canyon Creek area.	1,900	L, G, h	Gentle, W	B, A, T, F, C	Fairly moist but well drained.
Summerland	1,200	V	Gentle, SE.	B, M, S	Moist. Some springs. Well drained.
Oliver	1,000	V, S, P	Flat	S, A	Swampy in some places.
Near Osoyoos:					
Haynes Creek area.	2,500	V, S, L	do	A, C, B, Y, P, F, L.	Summer showers more frequent than at Oliver (nearest weather station).
Nine Mile Creek area.	3,200	V, S, H, p	do	Y, F, E, A, M, L.	Semiswampy in places. More rainfall than at Haynes Creek.
Camp McKinney	4,500	S, H, g	do	P, L, S, A	Much more rain than at Rock Creek (nearest weather station).
Coastal British Columbia:					
Garibaldi (Daisy Lake):[3]					
Chance Creek area.	1,100	S, H, g	do	W, H, F, A, C	
Lake area	1,100	S, H, L	do	W, F, A, C, H	
Near Mile 72, Pacific Great Eastern Ry.:					
Lower Trail area.	2,200	R, H, L	Steep, S	F, A, M, B	Moist in patches but well drained. Nearest weather station, Owl Creek.
Tenquil Valley area.	4,800	V, L	Steep, SE.	H, W, E, I, K, A.	Moist, swampy for *Ribes lacustre*. Well drained for *R. viscosissimum*. Nearest weather station is at Owl Creek.

[1] Key: G, gravel; H, humus; L, loam; P, peat; R, rocky; S, sand; T, silt; V, volcanic. Capital letters indicate abundance.

[2] Key: A, alder; B, birch; C, cottonwood; E, Englemann spruce; F, Douglas fir; G, swamp grasses; H, western hemlock; I, alpine fir; K, whitebark pine; L, larch; M, maples; P, lodgepole pine; S, willow; T, western red cedar; W, white pine; Y, yellow pine.

[3] Formerly Daisy Lake; name recently changed to Garibaldi.

two at higher elevations at the edge of the dry belt on Anarchist Mountain, east of Osoyoos, British Columbia (Haynes Creek and Nine Mile Creek). An additional area was established in 1927 near

Camp McKinney, several miles northeast of Nine Mile Creek. The last three areas, particularly the one at Nine Mile Creek, approach more closely the conditions of the interior white pine stands than do those at Kelowna, Summerland, and Oliver. Most of the white pine region plant species other than white pine are present, and the climate is fairly similar to that of much of the pine region.

During the course of these tests in the interior dry belt, a parallel series of studies involving species of ribes important in the coastal region of British Columbia was carried out at various natural infection centers in the latter region. Although *Ribes petiolare* and *R. inerme* do not occur there, *R. lacustre* and *R. viscosissimum* are native and were included at four of the study areas. At three of these areas, aeciospores from adjacent infected pines were so abundant that no inoculations were necessary because natural infection on the ribes approximated that produced by inoculation. In the fourth area, nearby aeciospore production was insufficient and the plants were inoculated. The studies of *R. lacustre* and *R. viscosissimum* in the coastal region served not only for comparison of the susceptibility of these two species occurring in the interior region, but also afforded an opportunity to observe the effect of somewhat different climatic conditions on the development of the rust on these two hosts tested in both regions.

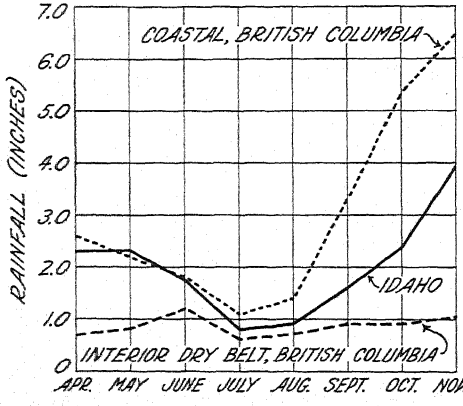


FIGURE 2.—Average rainfall during growing season. Graph prepared from United States and British Columbia weather records up to and including 1933. Records from the following stations were used. For Idaho: Avery, Potlatch, Prichard, St. Maries, Priest River Experiment Station, and Pierce. For interior British Columbia: Kelowna, Summerland, Oliver, and Rock Creek. For coastal British Columbia: Garibaldi, Owl Creek (Pemberton Hatchery), and Vancouver. The latter station has been included because rainfall there is about average for a rather large portion of the coastal region. Averages for the various stations represent periods ranging from 10 to 33 years.

A condensed description of the various areas is given in table 1. Rainfall during the growing season at weather stations nearest the study areas and at several points in the Idaho white pine region is shown graphically by months in figure 2. It will be seen that precipitation during this period was somewhat more abundant near the coast and in Idaho than in interior British Columbia. Relative humidities also averaged somewhat lower in the latter region. As will be shown later, however, these differences had little effect on the susceptibility of ribes to the rust.

INOCULATIONS AND EXAMINATIONS

The number of bushes of each species tested on areas in interior British Columbia is shown by years in table 2. These figures include the three growth forms—open, part-shade, and shade—of ribes studied. The studies involved 5,098 tests of a total of nearly 3 million leaves on 2,740 different bushes. Additional details regarding the basis for each species and form are included later under Results and Discussion.

TABLE 2.—*Bushes of Ribes species used each year in the tests on study areas in interior British Columbia* [1]

Species and area	Ribes bushes tested in—				
	1924	1925	1926	1927	1928
R. petiolare:	*Number*	*Number*	*Number*	*Number*	*Number*
Swamp	93	135	153	76	
Summerland			18	16	
Oliver			82		
Nine Mile Creek			148	144	145
Haynes Creek			96	50	
Camp McKinney				30	30
R. inerme:					
Swamp	90	104	166	66	
Oliver			66		
Nine Mile Creek			164	159	159
R. viscosissimum:					
Canyon Creek	99	98	184	280	150
Camp McKinney				100	100
R. lacustre:					
Swamp	52	24	48	47	
Canyon Creek	93	99	216	315	157
Nine Mile Creek			165	164	164
Haynes Creek			105	48	
Camp McKinney				100	100

[1] In the majority of cases the number of bushes tested in a given year includes some of those tested in a previous year or years.

All bushes tested in the interior dry belt and many of those on areas near the coast were artificially inoculated. Under overcast skies and when a good rain appeared imminent, the inoculum was applied to the under surface of the leaves with the aid of a paper bag. The bag was partly filled with freshly sporulating cankers and then used as a bellows for puffing the spores into the bushes. This method assured a fairly even distribution of spores over the leaves. Each bush was given a heavy shower of aeciospores, comparable, it was believed, to that which might have occurred naturally had the bushes been growing in close proximity to heavily infected pines. Inoculations were made in the spring, generally when ribes leaves were young and highly susceptible (*6*), and were repeated whenever weather immediately following the first inoculation was not favorable to infection. Leaf development and weather conditions accompanying each inoculation of plants in the interior are summarized in table 3.

The test plants were examined at fairly regular intervals during each growing season, data being taken as previously outlined under Development of Study Methods. During 1924 and 1925, system A (data by individual bushes) was used. Tests of greater numbers of plants during subsequent years necessitated the use of system B (estimates by groups) except in a few special instances where differences between individual bushes of the same species were being studied. During 1924, when data were recorded approximately once each month, great changes in extent and character of infection occurred between examinations. Since at that time relatively little was known about the development of the rust on these species of ribes, the interval between examinations was shortened to 1 or 2 weeks in 1925. In 1926 and 1927, data were taken every 3 or 4 weeks; and in 1928, every month or 6 weeks. Intensification of the rust by urediospores is irregular, new infection almost always developing only after rainy periods, but knowledge of the incubation period (*13, p. 40*), together

with a fairly complete record of weather conditions, generally made it possible to synchronize examinations with the culmination of each wave of intensification.

TABLE 3.—*Artificial inoculations of Ribes species on study areas in interior British Columbia, 1924–28*

Year	Areas inoculated	Date	Leaf development [1]				Weather following inoculations
			R. petiolare	*R. inerme*	*R. viscosissimum*	*R. lacustre*	
1924	Swamp and Canyon Creeks.	May 5–6	3	3	3	3	Warm and dry.
	do	May 16–17	4	4	4	4	Hot and dry.
	do	May 31					Showers in evening.
1925	do	May 18	3–4	4	4	4	Heavy dew but no rain until May 28.
	do	May 29					Good showers.
1926	do	May 4	4	4	4	4	Showers each night.
	Summerland and Oliver	May 5	4	4			Showers nights, May 5, 6.
	Nine Mile and Haynes Creeks.	May 6–7	[2] 3 (1–2)	3 (2)		3	Unsettled; rainy nights.
	All 1926 areas	May 10–12					Clear to cloudy; no rain until evening of May 13.
1927	Swamp and Canyon Creeks.	May 16	2 (3)	3 (3–4)	3–4	3–4	Good showers.
	Summerland	May 17	3				Do.
	Nine Mile and Haynes Creeks.	do	2	3–4	2	3–4	Do.
	Nine Mile Creek	June 5	3	4		4	Good showers June 7, 8.
	Camp McKinney	June 6	1–2		3–4	3	Good rain.
1928	Canyon Creek	May 11			4	4	Very little rain.
	do	May 21			5	4	Light shower.
	do	May 28			5	5	24 hours of rain.
	Nine Mile Creek	May 30	4	4		4	Rainy.
	Camp McKinney	do	1–3		3	3	Do.
	Nine Mile Creek	June 7	4	4		4	Thunder shower June 8.
	Camp McKinney	do	3		3	3	Do.

[1] Leaf-development stages are classified as follows: 1, Just breaking from bud; 2, up to ⅓ apparent full size; 3, up to ⅔ apparent full size; 4, oldest leaves have reached apparent full size; 5, many leaves have reached apparent full size and are beginning to harden.

[2] Numbers in parentheses refer to shade-form plants, other numbers to open form. Where no distinction is made, development was approximately the same in both forms.

RESULTS AND DISCUSSION

RIBES GROWTH FORMS

Test plants of each species were divided into three classes—open form, part-shade form, and shade form—data from which were kept separate throughout the study. Bushes entirely exposed to the sun during most of the day were classed as open form, while those receiving little or no direct sunlight made up the shade-form group. Part-shade plants were approximately intermediate in exposure to sunlight.

Since infection is favored by succulence of foliage (*2, p. 666; 6; 13, pp. 45–46*), the differences in susceptibility found between the three growth forms are largely explained by the differences in the character of their leaf development. Open-grown ribes are usually short and dense. Their leaves, which are relatively small and thick, generally become quite tough within 4 to 6 weeks after breaking from the buds. Shade-form plants are more rambling and loosely branched, with relatively large thin leaves. Although these leaves toughen to a certain extent, the process is slow and never reaches a degree comparable to that of open-form leaves (*6, p. 97*). Differences in time and degree of foliage maturation induced by exposure are very striking in both *Ribes inerme* and *R. lacustre*. Leaves on open and shade *R. petiolare*

differ appreciably, but infection does not seem to be affected by leaf character to the same extent here as in the other species. New leaves usually continue to appear on all species and forms until about the first of July, after which leaf production is negligible.

SEASONAL DEVELOPMENT OF THE RUST ON RIBES

Characteristic examples of the development of the rust are given graphically in figure 3, where percentages of total leaf surface infected and total leaf surface bearing telia are shown throughout the season for shade-form plants at Nine Mile Creek in 1926 and at Camp McKinney in 1928. Examinations are indicated by small circles, and the curves between examination dates were sketched from general knowledge of the relation between weather conditions and uredial intensification. Percentages at any point in figure 3 represent accumulations, to that date, of all infection, both living and dead, and the figures throughout the remainder of this paper are based on the accumulated totals of such infection at the end of each season.

These examples are fairly typical of the rust behavior on open-form as well as on shade-form bushes. It will be seen that during the early part of the season the disparities between species offer little or no indication of their relative susceptibility as it finally appears. The great bulk of the infection on both *Ribes petiolare* and *R. inerme* generally develops from late June to mid-August, through uredial intensification. In the other two species (*R. lacustre* and *R. viscosissimum*) this midsummer pyramiding of infection is usually relatively unimportant.

GENERAL SUSCEPTIBILITY OF THE FOUR SPECIES

Total average infection and telial production on all test bushes in interior British Columbia are shown in table 4 and figure 4. *Ribes petiolare* stands far ahead of the other species in both respects. *R. inerme*, also, is a very congenial host, but *R. viscosissimum* and *R. lacustre* are relatively resistant and the latter is particularly low in the production of telia. Differences between forms are as pronounced as those between species, shade and part-shade plants showing more infection and bearing more telia than corresponding open-grown bushes. Infection is probably facilitated by more favorable moisture conditions in the shade as well as by the greater succulence of shade-form leaves.

TABLE 4.—*Basis of tests and summary of infection on inoculated Ribes plants, interior British Columbia, 1924–28*

Species	Basis									Average total leaf surface[1]					
	Leaves tested			Bushes			Tests[2]			Infected			Bearing telia		
	Open form	Part-shade form	Shade form	Open form	Part-shade form[3]	Shade form	Open form	Part-shade form	Shade form	Open form	Part-shade form	Shade form	Open form	Part-shade form	Shade form
	No.	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*
R. petiolare	603, 533	121, 372	169, 004	234	150	346	381	250	585	22. 6	36. 1	34. 8	20. 3	35. 6	32. 7
R. inerme	366, 710	156, 363	188, 161	218	85	262	384	185	405	13. 9	22. 4	23. 0	4. 9	18. 8	18. 7
R. viscosissimum	89, 226	------	66, 159	256	----	269	507	----	504	6. 6	----	11. 5	3. 5	----	7. 3
R. lacustre	617, 127	235, 568	305, 309	306	132	482	687	259	951	3. 4	4. 3	8. 1	. 4	. 7	2. 9

[1] Averages computed on total-leaf basis.
[2] The same bush tested in each of 2 years equals 2 tests, tested 3 years equals 3 tests, etc.
[3] Tested during 3 years only (1926, 1927, and 1928).

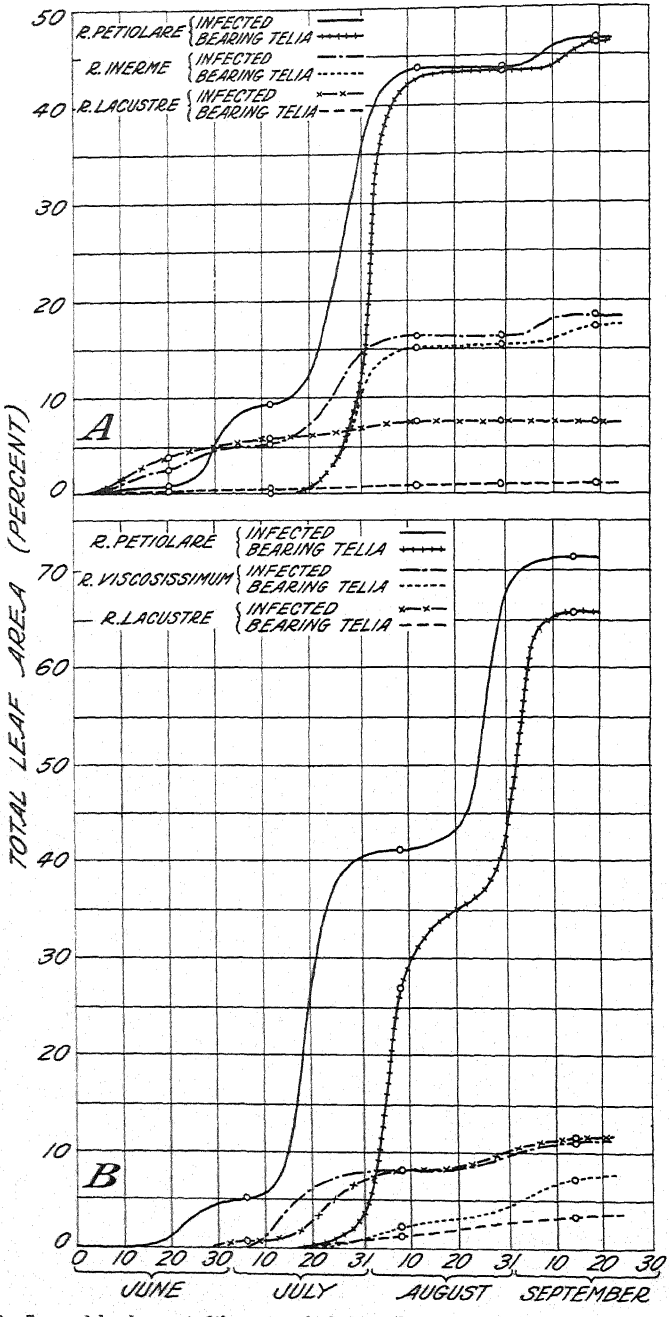


FIGURE 3.—Seasonal development of the rust on shade-form ribes. *A*, At the Nine Mile Creek area in 1926. Basis: *R. petiolare*, 50 bushes and 20,505 leaves; *R. inerme*, 50 bushes and 37,540 leaves; *R. lacustre*, 50 bushes and 35,950 leaves. *B*, At the Camp McKinney area in 1928. Basis: *R. petiolare*, 30 bushes and 4,800 leaves; *R. viscosissimum*, 50 bushes and 8,450 leaves; *R. lacustre*, 50 bushes and 14,400 leaves.

Data from 144 susceptibility tests, involving 34,208 leaves of *Ribes viscosissimum*, and from 1,214 tests, involving 420,756 leaves of *R. lacustre*, in the coastal region of British Columbia, generally substantiate the results obtained from the tests in the interior for these species. Many of the coastal inoculations were unsuccessful because of attendant unfavorable weather, but in all cases where either natural or artificial infection by aeciospores was secured under favorable conditions the final infection was approximately similar to that recorded in table 4. It therefore appears that the greater precipitation and higher relative humidity characteristic of the coastal region have little effect upon uredial intensification, and that the behavior of the rust on ribes within the commercial range of western white pine, where spring and summer moisture conditions are roughly intermediate between

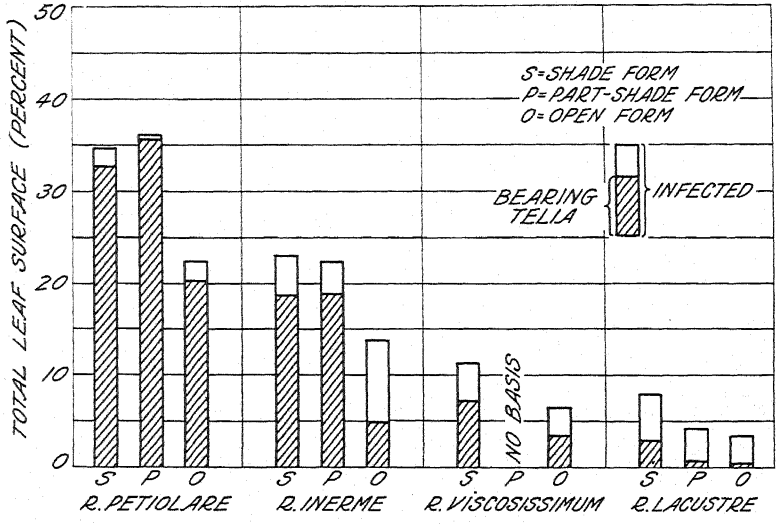


FIGURE 4.—Average infection and telial production on ribes tested in interior British Columbia.

those of the interior and coastal test areas, will not differ significantly from that herein described. This assumption is substantiated by preliminary results from the north Idaho pine region, which are presented in table 5.

The Idaho infection averages for *Ribes petiolare* are similar to the corresponding British Columbia averages, but those for *R. viscosissimum* and *R. lacustre* are somewhat lower than the ones in British Columbia. The Idaho data are for 2 years only, but on this basis, together with observations made at several other localities in the State during 5 seasons, it is believed that ribes infection will not vary greatly from that in British Columbia. The Idaho studies included 562 tests involving nearly 400,000 leaves

TABLE 5.—*Infection on naturally infected Ribes, Idaho, 1933 and 1934* [1]

Place and area	Species	Year	Basis: Bushes: Open form	Basis: Bushes: Part-shade form	Basis: Bushes: Shade form	Basis: Leaves: Open form	Basis: Leaves: Part-shade form	Basis: Leaves: Shade form	Total leaf surface: Infected: Open form	Total leaf surface: Infected: Part-shade form	Total leaf surface: Infected: Shade form	Total leaf surface: Bearing telia: Open form	Total leaf surface: Bearing telia: Part-shade form	Total leaf surface: Bearing telia: Shade form
			No.	*No.*	*No.*	*No.*	*No.*	*No.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*
Fernwood, Crystal Creek.	*R. petiolare*	1933	20	25		24,510	14,215		34.40	40.20		33.80	39.50	
		1934	20	25		15,145	8,950		12.09	27.17		11.99	27.14	
	R. viscosissimum.	1933	25	25	25	4,195	5,045	7,785	.53	1.01	0.66	.17	.52	0.61
		1934	25	25	25	5,761	4,920	11,878	.42	.83	.76	.36	.79	.74
	R. lacustre	1933	25	25	25	51,895	29,645	33,725	.18	.73	2.42	.02	.26	1.04
		1934	25	25	25	44,095	62,885	33,135	.03	.11	.72	.004	.04	.44
Elk River, Ruby Creek.	*R. lacustre*	1933		25			6,725			2.06			.70	
		1934		25			13,351			3.60			.99	
	do	1933		25			2,289			6.66			4.83	
		1934		22			3,571			.92			.48	
Clarkia, St. Maries River.	*R. petiolare* [2]	1933		25			3,625			36.00			32.90	
	R. inerme [2]	1933		25			3,250			7.50			4.68	
	R. lacustre [2]	1933		25			5,875			3.30			1.08	

[1] All the ribes plants for which data are given were growing in close proximity to heavily infected pines.
[2] Only 1 examination was made of these bushes, on Aug. 17. Shortly after that date the bushes were destroyed by blister-rust eradication crews. No doubt considerably more rust would have developed on them, for intensification of the disease was taking place in this vicinity until about the first part of October. Data were taken on all the other groups for both years until intensification had ceased.

REACTION OF INDIVIDUAL BUSHES WITHIN GROUPS

Individual ribes bushes of a given species frequently show a very marked difference in their reaction to white-pine blister rust. The extent of this variation, as illustrated by the differences in percentage of leaf surface infected within groups of the four species and their forms under consideration, is indicated in table 6, which shows the number of bushes of each group in the different infection classes.

The individuals composing each group of these bushes occurred within small limits, the radii of which seldom exceeded 50 feet. On each area the ground was nearly level and in general the environmental conditions to which the bushes were subjected appeared fairly uniform. There was no evidence, therefore, that variations in environment were great enough to affect significantly the reactions of the individual bushes to the rust.

Practically all infection estimates during and after 1926 were made by groups rather than by individual bushes; data are therefore presented for only the 1924 and 1925 tests. However, since no bush was used more than once during the 2 years, these data are based on a good assortment of individuals. They may be considered fairly representative of the degree of variability usually encountered within test groups throughout the study, in spite of the fact that infection averages differed appreciably from the final figures for all groups as shown in table 4.

Within the groups represented in table 6, individual infection ranged from less than 1 percent of the leaf surface in all forms to about 70 and 75 percent, respectively, for the open and shade forms of *Ribes petiolare;* 75 and 90 percent, respectively, for the open and shade forms of *R. inerme;* 45 and 70 percent in *R. viscosissimum;* and about 10 and 70 percent in *R. lacustre.* These maxima are in most cases still considerably below the greatest actually recorded during

TABLE 6.—*Variation of the extent of infection within groups of inoculated Ribes, interior British Columbia, 1924 and 1925*

Leaf surface per bush infected (percent)	Bushes of species and forms tested falling in the indicated susceptibility class																				
	R. petiolare					*R. inerme*						*R. viscosissimum*				*R. lacustre*					
	1924		1925			1924		1925				1924		1925		1924			1925		
	Open	Shade	Open		Shade	Open	Shade	Open		Shade		Open	Shade	Open	Shade	Open	Shade		Open	Shade	
			No. 1	No. 2				No. 1	No. 2	No. 1	No. 2						No. 1	No. 2		No. 3	No. 4
	No.	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*
0	0	11	1	0	0	0	0	0	0	0	0	0	1	0	0	0	0	2	0	0	0
Trace–1	5	5	16	0	10	1	0	0	4	1	3	5	8	14	2	19	1	24	38	1	17
1.01–5	3	3	3	3	32	0	1	6	3	7	1	12	15	17	12	6	3	23	10	6	17
5.01–10	2	8	0	1	6	0	0	11	3	7	7	7	12	11	8	2	5	7	1	4	7
10.01–15	0	4	1	2	1	0	2	3	1	7	1	5	5	6	5	0	3	3	0	3	6
15.01–20	0	5	1	3	0	1	4	2	0	7	1	5	3	1	7	0	2	5	0	4	1
20.01–25	0	2	0	3	0	1	3	3	2	1	0	4	5	1	2	0	10	1	0	2	0
25.01–30	2	1	1	8	0	2	4	1	0	2	0	0	1	0	5	0	2	0	0	2	0
30.01–35	2	1	5	7	0	6	8	2	0	1	0	2	5	0	2	0	6	1	0	2	1
35.01–40	6	2	5	3	0	5	13	2	0	1	0	0	0	0	1	0	5	0	0	0	0
40.01–45	6	2	6	1	0	1	6	0	0	0	0	1	1	0	2	0	8	0	0	0	0
45.01–50	1	1	3	2	0	3	3	0	0	1	0	0	1	0	1	0	4	0	0	0	0
50.01–55	5	0	3	4	0	2	1	1	0	0	0	0	0	0	0	0	1	0	0	0	0
55.01–60	6	3	1	1	0	1	2	2	0	0	0	0	1	0	0	0	1	0	0	0	0
60.01–65	3	0	2	0	0	1	4	0	1	0	0	0	0	0	0	0	0	0	0	0	0
65.01–70	3	0	0	0	0	1	5	0	0	0	0	0	0	0	1	0	1	0	0	0	0
70.01–75	0	1	0	0	0	2	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0
75.01–80	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0
80.01–85	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
85.01–90	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
90.01–95	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
95.01–100	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Total	44	49	48	38	49	27	63	33	14	35	13	41	58	50	48	27	52	66	49	24	49
Average percentage of leaf surface infected [1]	*Pct.* 39.4	*Pct.* 20.1	*Pct.* 24.3	*Pct.* 29.7	*Pct.* 2.5	*Pct.* 41.4	*Pct.* 43.8	*Pct.* 17.3	*Pct.* 10.6	*Pct.* 13.5	*Pct.* 6.1	*Pct.* 10.4	*Pct.* 12.4	*Pct.* 5.0	*Pct.* 16.4	*Pct.* 1.1	*Pct.* 29.6	*Pct.* 4.6	*Pct.* 0.7	*Pct.* 13.1	*Pct.* 4.6

[1] Averages computed on bush basis.

the course of this study. Individual plants of *R. petiolare* have frequently been observed with over 90 percent of the leaf surface infected. In one case at Camp McKinney, in 1928, a group of 30 shade-form bushes of *R. petiolare* averaged 71.3 percent of the leaf surface infected. The shade and part-shade forms of this species have already been shown by the averages in table 4 to be the most susceptible of all the species and forms represented in the tests. The data in table 6 for the shade-form group tested in 1925 are therefore decidedly atypical.

The maxima for *Ribes inerme* in some of the groups other than those at present under consideration sometimes exceeded 90 percent of the surface infected, although the leaves of this species are much more susceptible to injury from the rust than are those of *R. petiolare*, and usually wilt and fall to the ground before such a degree of infection is reached. Occasional bushes of *R. viscosissimum* and the shade form of *R. lacustre* also ranged considerably higher in percentage of leaf surface infected than shown, while bushes of the open form of *R. lacustre* frequently exceeded the indicated maximum by large margins. For group 1 of the shade form of *R. lacustre* in table 6, however, the average degree of infection and the extent to which the bushes range into the heavier infection classes are far greater than usually encountered in this form and species.

The distribution of the samples of *Ribes petiolare* shown in table 6 indicates that this species is made up of at least two strains, one resistant and the other highly susceptible. Because of insufficient data, the values in this table do not give a correct impression of the proportion in which the resistant strain occurs. While exact figures are not available, observational experience throughout the study indicated that not more than 10 or 15 percent of the bushes possessed resistance of that order, the rest ranging mainly from moderate to very high in susceptibility.

Ribes inerme (table 6) shows a similar though less marked tendency toward division into strains. *R. viscosissimum* exhibits a dispersion of the bushes over a considerable range of susceptibility, with a concentration in the resistant classes. The open form of *R. lacustre* shows a characteristic concentration of the bushes in the extremely resistant classes. Disregarding the atypical case of group 1, which has already been mentioned, the shade form shows a typically light infection, although individual bushes may exhibit a fairly high susceptibility.

General observations indicated that the technique employed in these tests largely, if not entirely, eliminated the effect of irregularities in the inoculations. Infection resulting directly from aecial inoculations is of relatively small extent in all species (*6, p. 109*) (see also fig. 3), the great bulk of infection on the susceptible plants developing later in the season by means of uredial intensification. This is usually the case even when bushes are showered with great quantities of aeciospores. All of the groups now being considered, and practically all of the other plants tested during the study, were inoculated more heavily than necessary to secure approximately maximum initial infection. Therefore, and since pains were taken to secure as even distribution of the inoculum over the foliage as possible, it may be assumed that variability of individual bushes in extent of infection was not the result of inequalities in inoculation.

It will be seen (table 6) that for no species can infection be considered approximately uniform, even when bushes are subject to the same general environment. Because of this variability, results secured from small numbers of bushes are extremely unreliable indices of general susceptibility. When the effects of dissimilar environments and wide local and annual differences in weather are considered, it is obvious that a large basis is necessary for the determination of average susceptibility.

INHERENT DIFFERENCES WITHIN SPECIES

Evidence of inherent differences in susceptibility between bushes of the same species, particularly *Ribes petiolare*, has already been pointed out in the discussion of table 6. The extremely light infection of the shade group of this species at the Kelowna swamp in 1926 and 1927 may be considered as additional evidence of the existence of resistant strains in this species.[7] Besides this group, there also occurred at this same area the five groups of *R. petiolare* included in table 6 and three others composed of open-form bushes, or a total of nine separate groups. With the exception of one very lightly infected group, all open-form groups became abundantly infected every year they were tested, while only one of the shade-form groups developed infection enough to be termed moderate in amount. In every group, however, there were resistant and susceptible individuals. Some of these individuals were inoculated as many as 4 years and consistently maintained their resistance or susceptibility during that time. Also, at all the other areas where *R. petiolare* occurred there were individuals exhibiting these characteristics, and this was true for the other species tested at the various localities.

All nine groups of *Ribes petiolare* mentioned above occurred on a relatively small portion of a large level swamp formed by the gradual recession of a lake. The resistance shown by the shade group in 1926 and 1927, therefore, cannot be ascribed to a difference in site factors but must be considered as probably inherent. Individuals in this group, in many cases apparently derived by layering from a common origin, generally became infected either very lightly or not at all, while others, closely associated or even intermingled with the resistant bushes, were much more severely attacked. Hahn (*2, table 2*) records the occurrence of several immune plants of *R. petiolare* in a series tested in the greenhouse, and in another publication (*3*) clearly demonstrates that wide differences in susceptibility exist between horticultural varieties of both *R. nigrum* L. and *R. sativum* Syme.

In an effort to obtain additional information regarding the extent of inherent differences in susceptibility within the same species, a number of bushes previously found to range from resistant to highly susceptible were selected from each species and form and tested during a period of 2 years or more. Each group was composed of plants growing fairly close to each other and under environmental conditions as similar as possible. Results are illustrated in figures 5 and 6, where data from four representative groups are presented graphically.

[7] See section entitled "Local and annual differences in infection."

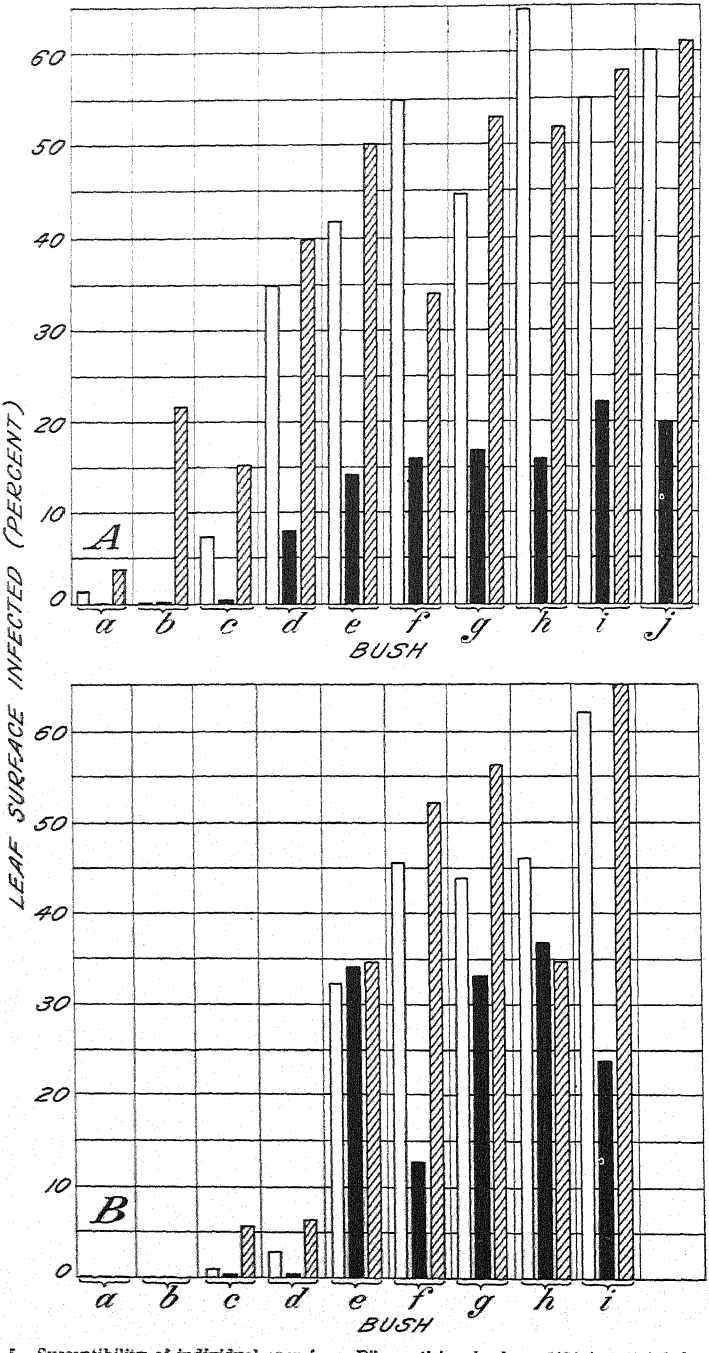


FIGURE 5.—Susceptibility of individual open-form *Ribes petiolare* bushes. 1924 (or 1925) infection, unshaded; 1927 infection, solid; 1928 infection, diagonally hatched. Two separate groups of bushes (*A*, *a–j*, and *B*, *a–i*), are represented in the figure. These groups occurred about 150 yards apart within a large swampy area.

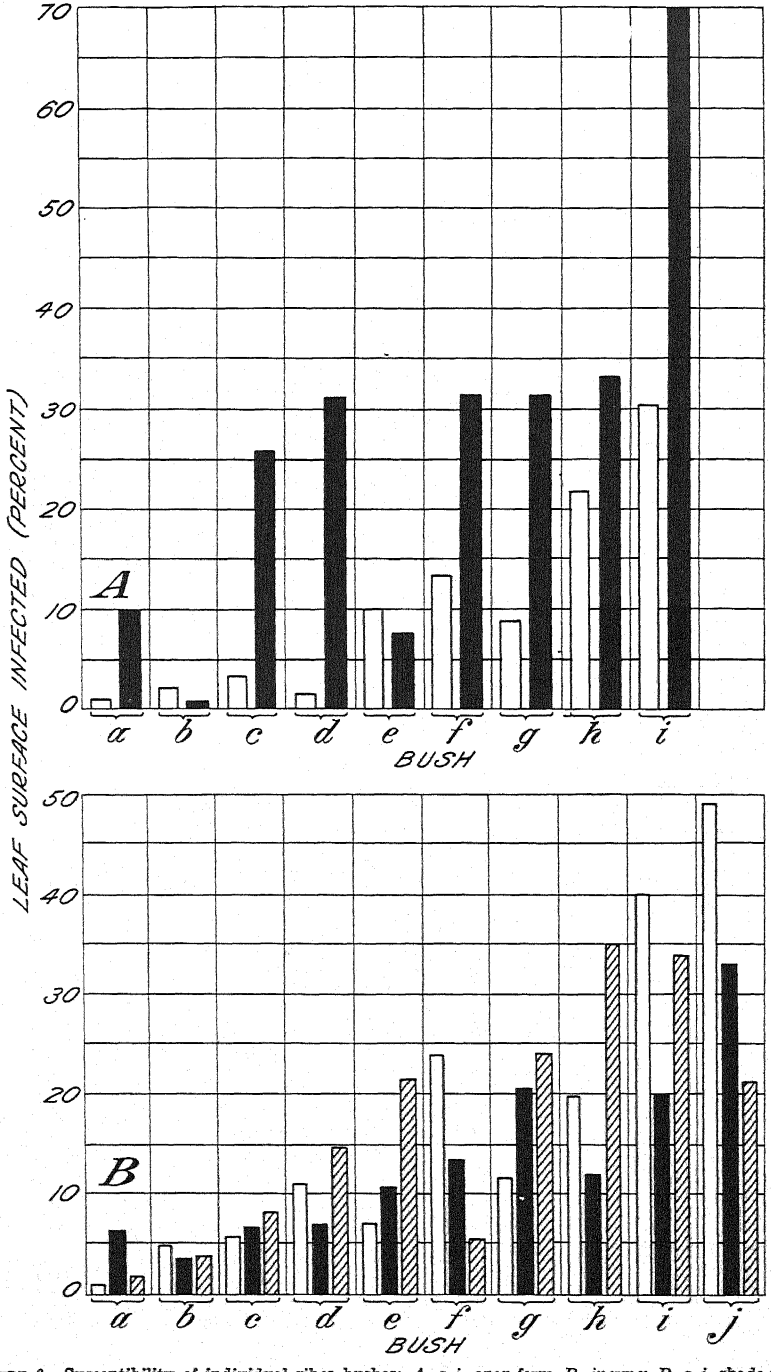


FIGURE 6.—Susceptibility of individual ribes bushes: *A*, *a–i*, open-form *R. inerme*; *B*, *a–j*, shade-form *R. viscosissimum*. 1925 infection, unshaded; 1927 infection, solid; 1928 infection, diagonally hatched.

While differences were far from being entirely consistent, in general a bush severely infected during one year also displayed relatively high susceptibility during the other years. Likewise a considerable proportion of the bushes exhibiting extreme relative resistance within a group did so consistently.

In general, *Ribes petiolare* was of uniformly high susceptibility and but little affected by weather conditions; however, the small proportion of the bushes of this species that were resistant were extremely and consistently so. The apparently low susceptibility of bushes *a*, *b*, and *c* in figure 5, *A*, and *a*, *b*, *c*, and *d* in figure 5, *B*, as compared with that of the remaining bushes in these groups, tends to confirm the evidence of the existence of a resistant strain or strains. In the other three species there are less definite indications of inherent intraspecific differences in susceptibility, and any such differences as may occur are apparently not so great as in *R. petiolare;* the available evidence (e. g., fig. 6, *B*) suggests, however, that even here they may be of appreciable magnitude.

DEVELOPMENT OF TELIA BY SPECIES

The proportions of the infected leaf surface which bore or for various reasons failed to bear telia are shown for the different species and their forms in table 7. *Ribes petiolare* and the shade and part-shade forms of *R. inerme* developed telia over most of the infected surface, while *R. lacustre* and the open form of *R. inerme* were much lower in the production of telia. *R. viscosissimum* was intermediate between the two types.

TABLE 7.—*Percentage of infected leaf surface which bore or failed to bear telia on inoculated Ribes, interior British Columbia, 1924–28*

Species	Infected leaf surface [1]—											
	Bearing telia			Bearing uredia but dead before producing telia			Lost through partial defoliation [2]			Necrotic		
	Open form	Part-shade form [3]	Shade form	Open form	Part-shade form [3]	Shade form	Open form	Part-shade form [3]	Shade form	Open form	Part-shade form [3]	Shade form
	Per cent	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*
R. petiolare	89.8	98.7	94.0	6.0	0.7	1.5	3.0	0.4	4.2	0.6	0.1	[4] 0.2
R. inerme	35.2	84.0	81.3	24.9	5.7	8.3	34.2	7.8	8.2	5.0	2.2	2.0
R. viscosissimum	53.0	-----	63.5	29.4	-----	19.2	8.4	-----	5.6	7.3	-----	9.7
R. lacustre	11.8	16.3	35.8	79.8	80.2	46.6	3.2	0.3	4.4	5.1	2.8	10.0

[1] Basis, all plants tested, as in table 4.
[2] Includes natural defoliation as well as that induced by rust and drought before telia could be produced.
[3] Tested during 3 years only, 1926–28.
[4] In most cases insignificant percentages of the infected leaf surface still bore uredia at the time of final examination; consequently the percentages shown here usually total slightly under 100 for each form of each species.

Table 7 indicates that necrosis, i. e., death of infected leaf surface before either fruiting stage appears, was of comparatively little significance and that only in the case of open-grown *R. inerme* was falling of infected leaves from the bushes an appreciable factor in the reduction of telial sporulation. Most of the difference between infection and telial production is accounted for by death of leaf surface

bearing uredia. This occurred most commonly in the species and forms of relatively low telial production and was most striking in the case of *R. lacustre*, where it was apparently intensified by the warmth and dryness of midsummer. The great majority of the telia produced by this species developed at a relatively early date. Because of the partial loss of infected leaves in the natural cast of early-formed foliage and the failure of the rust to spread to other leaves, it is often difficult during the latter part of the growing season to find the rust on *R. lacustre*, even on bushes initially well infected.

Telial production per unit of leaf surface infected showed a general tendency in the present tests to increase with the percentage of leaf surface infected (table 8). This relationship was not sufficiently constant, however, to permit the certain prediction that because a species exhibits high susceptibility to infection it will have a similarly high percentage of the infected surface bearing telia, or vice versa. Variation in this relationship is evident between the species and forms shown. For example, the open form of *Ribes inerme*, with an average of nearly 14 percent of the leaf surface infected, bore telia on only about 35 percent of the infected surface, while the shade form of *R. viscosissimum*, with only 11.5 percent of the surface infected, bore telia on 63.5 percent of the infected surface. Similarly, there are wide discrepancies between production of telia on the various forms of *R. lacustre* and on the open form of *R. viscosissimum*, which falls in the same range as *R. lacustre* in percentage of leaf surface infected. Mielke and Hansbrough (*8*) found a far more extreme example of lack of correlation in the case of *R. roezli* (Reg.) Cov. and Brit., one of the most important California species. Tests of this species over a period of 2 years gave an average of about 46 percent of the leaf surface infected, while only about 0.7 percent of the leaf surface bore telia. In this case infection hastened defoliation of the test plants to such a degree that most of the infected leaves fell before telia could be produced. This species was tested outside its range, however, and may possibly show a considerably higher telial productivity in California.

TABLE 8.—*Percentage of leaf surface infected in relation to percentage of leaf surface bearing telia on inoculated Ribes, interior British Columbia, 1924–28*

Species and growth form	Leaf surface infected	Infected surface bearing telia	Species and growth form	Leaf surface infected	Infected surface bearing telia
	Percent	*Percent*		*Percent*	*Percent*
R. petiolare (part shade)	36.1	98.7	*R. viscosissimum* (shade)	11.5	63.5
R. petiolare (shade)	34.8	94.0	*R. lacustre* (shade)	8.1	35.8
R. inerme (shade)	23.0	81.3	*R. viscosissimum* (open)	6.6	53.0
R. petiolare (open)	22.6	89.8	*R. lacustre* (part shade)	4.3	16.3
R. inerme (part shade)	22.4	84.0	*R. lacustre* (open)	3.4	11.8
R. inerme (open)	13.9	35.2			

Thus, while there is a general tendency toward positive correlation between percentage of leaf surface infected and percentage of infected surface bearing telia, this correlation is by no means absolute. The leaf tissues of *Ribes petiolare* are comparatively thick and strong and, once infected, are generally capable of sustaining and permitting the growth of rust hyphae until a good crop of telia is produced. The shade and part-shade forms of *R. inerme* are also high in the percent-

age of infected surface bearing telia, but the open form is relatively low because of the susceptibility of its tissues to rust and drought injury. *R. viscosissimum*, while relatively low in susceptibility, is relatively high in the percentage of the infected surface bearing telia. The tissues of this plant are more resistant to rust and other injury than are the open form of *R. inerme* and all forms of *R. lacustre*. The latter species produces telia from relatively small proportions of the infected surface, although under favorable conditions the shade form and, to a lesser degree, the part-shade form sometimes show a good production of telia. The open form is characteristically meager in telial production.

TELIOSPORE PRODUCTON BY SPECIES

There are great differences between species in teliospore production per unit of leaf area bearing telia. In some cases telial columns are small and sparse, while in others they are comparatively large and so abundant as to be almost matted. The latter is frequently the case with *Ribes petiolare*, the infected surface of which characteristically produces a relatively dense stand of well-developed telial columns. The distribution of the columns is somewhat sparser on the infected surface of *R. inerme*, but the columns are frequently longer, particularly on the shade and part-shade forms. Very short stout columns are commonly noted on *R. viscosissimum*, and their distribution is usually more scattered than on *R. inerme*. Telial development on *R. lacustre* in nature is almost always relatively sparse, and the columns are generally small in diameter although often quite long. Data in table 4 are therefore unsatisfactory as indices of teliospore production.

Taylor (*18*) has made counts of telial columns and teliospores for several ribes species, including one (*Ribes lacustre*) involved in the present study. Her results, unfortunately, are not generally applicable, since the averages given were determined for units of total leaf surface rather than infected or telium-bearing surface. In the present study, relative values roughly expressing the difference in teliospore production per unit of leaf surface bearing telia were secured by ocular estimate of the average relative density and bulk of the telial columns on the telium-bearing surface for each ribes species and form. These estimates, based on comparative study of the extensive collections of representative specimens taken during the tests, and relative values indicative of the actual differences in teliospore production between the various species and forms, are given in table 9.

Other studies not yet reported have demonstrated that either *Ribes viscosissimum* or *R. lacustre* is quite capable of spreading enough infection under natural conditions to destroy associated pine. The enormous potential pine-damaging power of *R. petiolare* and *R. inerme* may therefore be readily inferred from the comparative ratings in table 9.

TABLE 9.—*Relative teliospore-production estimates for inoculated Ribes species, interior British Columbia, 1924–28*

RELATIVE PRODUCTION OF TELIOSPORES PER UNIT OF TELIUM-BEARING SURFACE [1]

Species	Form of species		
	Open	Part shade	Shade
R. petiolare	100	85	75
R. inerme	60	70	80
R. viscosissimum	10		15
R. lacustre	5	6	7
RELATIVE TELIOSPORE PRODUCTION PER UNIT OF TOTAL LEAF SURFACE [2]			
R. petiolare	2,030	3,026	2,452
R. inerme	294	1,316	1,496
R. viscosissimum	35		109
R. lacustre	2	4	20

[1] Arbitrary scale: Relative mass of telial columns per unit of leaf surface bearing telia=100 in open-grown *R. petiolare*.

[2] The numbers given are relative values indicative of the actual differences in teliospore production between the various species and forms. The values were derived by multiplying average percentages of leaf surface bearing telia in table 4×100×the corresponding estimates shown in the upper part of table 9.

LOCAL AND ANNUAL DIFFERENCES IN INFECTION

The relative susceptibility and telial productivity of these four species and their forms in nature are fairly constant, and the average susceptibility of any one of them in Idaho seems to be about the same as in British Columbia despite some rather distinct climatic differences between the two regions. (See fig. 2, tables 4 and 5, and discussion.) There are, however, pronounced local and annual variations in the amount of infection in response to differences in the weather. An idea of the range of such variability may be gained from table 10, in which infection on groups of bushes in several different localities is shown for 2 or 3 consecutive years. In each locality the same groups of bushes were used during each year for which results are shown.

For the best initial infection from aeciospore inoculations in nature two conditions were found to be essential in the present tests: (1) That the leaves be young, which is their most susceptible stage (*6*), and (2) that favorable moist periods occur immediately following the inoculations. For uredial intensification thereafter a good distribution of favorable moist periods in synchronization with the production of urediospores is more important than the total amount of precipitation. Weather conditions during the 3 years differed widely in these respects.

Spring in 1926 was one of the earliest on record, stimulating an exceptionally early vegetative development of ribes. Favorable infection weather during the early part of the season, but mainly after the leaves had passed their most susceptible stages, was followed by severe drought which began in early June and continued into the latter half of August. In 1927 the season was late, with a corresponding retardation of the commencement of ribes leaf development. Early in the season moisture conditions were favorable, with precipitation above normal in May and early June. From then through July precipitation was somewhat below normal but well distributed, and there-

after it was considerably above normal as well as favorably distributed. Spring in 1928 was late, as in 1927, but most of May was relatively dry. Rains commenced in the latter part of May and from then into July precipitation was well above average and well distributed. This early-summer moist weather was succeeded by subnormal rainfall in August and by extreme drought during the remainder of the season. Monthly rainfall records covering the period from April to November at weather stations nearest the study areas are given for 1926 to 1928, inclusive, in table 11. These records have been compiled from daily weather reports which were used in the analysis of the above-mentioned infection conditions.

TABLE 10.—*Local and annual variation in severity of infection of Ribes species, British Columbia, 1926–28*

Growth form, species [1] and locality	Total leaf surface [2]— Infected			Producing telia		
	1926	1927	1928	1926	1927	1928
Open form:						
R. petiolare:	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
Kelowna swamp	27.9	40.2	----	27.8	36.7	----
Nine Mile Creek	51.4	35.0	3.0	51.0	34.3	3.0
R. inerme:						
Kelowna swamp	1.9	44.6	----	1.9	14.7	----
Nine Mile Creek	5.5	21.4	10.0	3.4	1.8	3.6
R. viscosissimum:						
Canyon Creek	.6	12.4	17.1	.2	3.3	4.7
Camp McKinney	----	7.5	.7	----	6.5	.4
R. lacustre:						
Canyon Creek	.05	13.6	3.9	Trace	.8	.5
Nine Mile Creek	.9	4.9	.7	.1	.4	.2
Camp McKinney	----	12.6	8.7	----	1.4	1.8
Shade form:						
R. petiolare:						
Kelowna swamp	.03	1.5	----	.03	1.4	----
Summerland	30.0	46.6	----	29.8	38.0	----
Haynes Creek	20.7	37.0	----	20.5	35.3	----
Nine Mile Creek	46.5	36.8	20.0	46.3	36.4	19.6
Camp McKinney	----	65.7	71.3	----	63.0	65.4
R. inerme:						
Kelowna swamp	21.1	34.4	----	21.1	30.1	----
Nine Mile Creek	18.7	29.1	10.4	17.3	20.5	8.2
R. viscosissimum:						
Canyon Creek	.9	11.8	22.9	.3	4.7	6.9
Camp McKinney	----	12.3	10.9	----	11.4	7.5
R. lacustre:						
Kelowna swamp	.01	7.2	----	Trace	3.5	----
Canyon Creek	.1	19.8	4.6	Trace	2.3	.6
Haynes Creek	2.1	13.8	----	.4	6.4	----
Nine Mile Creek	7.5	10.2	1.0	1.1	3.4	.3
Camp McKinney	----	13.3	11.2	----	8.9	3.4

[1] The number of bushes used as a basis in each test ranged from 16 to 64 but was usually about 50.
[2] Leaders indicate that the group was not tested.

Weather at the time of inoculation in 1926 was favorable to infection, but ribes leaves were too far advanced (*6*) for best results except at the Nine Mile Creek and Haynes Creek areas. In these two localities the development of vegetation was 7 to 10 days behind that at lower elevations and ribes leaves had not passed beyond the highly susceptible stage at the time of inoculation. Midseason intensification was limited by the decidedly subnormal rainfall during June and July.

TABLE 11.—*Rainfall* [1] *near study areas during growing season, interior British Columbia, 1926–28*

Locality and year	April	May	June	July	August	September	October	November
Kelowna:	*Inches*	*Inches*	*Inches*	*Inches*	*Inches*	*Inches*	*Inches*	*Inches*
1926	0.58	1.13	0.33	0.20	1.20	0.96	1.08	1.49
1927	.23	.81	.96	.33	1.97	2.71	1.76	2.95
1928	1.34	.82	1.86	.64	.82	.10	.49	.57
Normal	.73	.79	1.03	.56	.84	1.05	1.12	1.14
Summerland:								
1926	.83	.98	.69	.16	.83	.55	.73	.72
1927	.28	.82	.90	.54	1.37	2.17	1.34	2.57
1928	1.57	1.16	1.48	1.65	.23	.01	.39	.35
Normal	.72	.75	1.03	.51	.69	.78	.89	1.02
Rock Creek:								
1926	.76	1.31	1.53	.25	2.16	2.15	1.44	1.54
1927	.59	1.62	1.93	.50	1.25	4.06	1.22	2.61
1928	1.40	.40	3.76	1.73	.52	.00	.42	.42
Normal	1.00	1.18	1.83	.98	.96	1.09	.89	1.19
Oliver:								
1926	.33	.48	.49	.24	1.43	1.36	.94	.85
1927	.23	.77	1.55	.20	.96	1.62	.73	1.59
1928	1.00	.58	1.31	1.00	.28	.00	.23	.50
Normal	.43	.45	.97	.26	.43	.68	.67	.90

[1] From British Columbia Department of Agriculture weather reports.

The season of 1927 was outstandingly favorable for the development of the rust over most of the region. A good synchronization of the early susceptible stages in ribes leaf development and suitable weather generally attended the inoculations, resulting in good initial infection, and the well-distributed precipitation that followed encouraged an abundant uredial intensification. Mild rains from May 17 to June 13 stimulated the development of the rust on *Ribes lacustre* particularly, since any decided increase of infection in this species is limited, by the nature of its susceptibility, to the early part of the season. The only test groups on which infection was less severe than in 1926 were at Nine Mile Creek, where both open and shade forms of *R. petiolare* showed relatively less rust in 1927. Growth starts several days later in this species than in associated ribes, and by the middle of May, at the time of the first inoculations in 1927, only a few small leaves had appeared on the Nine Mile Creek bushes. A second inoculation in early June was not immediately followed by rain, and the initial infection resulting from the inoculations was therefore relatively light. Although infection intensified well later in the season, it did not entirely overcome its original handicap on these plants. *R. inerme* is very sensitive to weather conditions, and the open form of this species suffered so much defoliation during a local midseason heat wave at Nine Mile Creek that very little infected surface was left to produce telia.

In 1928 a considerable wait was required before weather favorable for inoculations arrived, and by that time, in late May and early June, the leaves had generally passed their most susceptible stages except at Camp McKinney. The plants at Canyon Creek were inoculated three times, and although many of the leaves were maturing and becoming resistant, infection was facilitated by showers following two of these inoculations. The rust developed particularly well there on *Ribes viscosissimum*. In this species, age of leaves at time of initial infection exercises less influence than in any of the other three ribes species (*6*). *R. lacustre*, the only other species tested

there, showed a decided reduction in degree of infection compared with that which it developed in 1927. Leaves of all the ribes were still fairly susceptible at Camp McKinney, but were becoming resistant at Nine Mile Creek, and therefore relatively little infection developed at the latter place in spite of favorable weather conditions following two inoculations. The prolonged dry spell beginning in late summer reduced subsequent intensification over the entire region, having an exceptional effect in the case of open-grown *R. petiolare* at Nine Mile Creek, where leaves, normally susceptible throughout their life, became leathery and resistant. Because of the importance of late pyramiding of infection in this species, the drought exercised an extremely retarding influence in this case.

Another group which exhibited an extraordinarily wide reduction from 1927 levels at Nine Mile Creek was the shade form of *Ribes lacustre*. By the time of the inoculations here in 1928, the leaves of this form had hardened off to a stage of such resistance that there was comparatively little initial infection or subsequent intensification.

Still another group to show a reduction in infection from 1927 levels was the open form of *Ribes viscosissimum* at Camp McKinney. This was surprising in view of the fact that the open form of *R. lacustre* there showed but a relatively slight reduction. Initial infection was lighter on both groups than in 1927, but a good wave of intensification raised the percentage of surface infected on the *R. lacustre* while the rust on *R. viscosissimum* remained practically at a standstill.

Considering the species individually (table 10), *Ribes petiolare*, with the exception of the open form at Nine Mile Creek in 1928, showed the smallest percentage of seasonal variation in the degree of infection. In this case the resistance was clearly induced by an extraordinary effect of the weather on the host. The shade-form plants of *R. petiolare* at the Kelowna swamp showed high resistance in both 1926 and 1927, while the open-form plants were heavily infected in both years. Here the resistance appeared to be primarily inherent, for conditions for infection in the shade at this place were favorable in both years, as indicated by the reaction of the shade-form plants of *R. inerme* nearby. *R. petiolare* as a rule maintained a high degree of susceptibility and was consistently high in the proportion of its infected surface that bore telia. This was true whether resistance appeared inherent, as in the case of the shade form at the Kelowna swamp, or induced, as in the case of the open form at Nine Mile Creek in 1928. Observations of the part-shade form of this species, aside from the results under immediate consideration, indicate that its reactions are similar to those of the shade form.

The shade form of *Ribes inerme* was generally similar to that of *R. petiolare* in uniformity of local and seasonal infection and in the ratio of the percentage of surface bearing telia to the percentage of surface infected. At the same time its open form in the same places and years was highly variable in both respects (table 10). The very light infection of this form at the Kelowna swamp in 1926, as compared with the heavy infection there under the more favorable weather conditions of 1927, and the low proportion of infected leaf surface bearing telia on the Nine Mile Creek plants in 1927, as compared with the relatively high proportion bearing telia on these plants under the lighter infection conditions of 1926, clearly illustrate this

point. As already shown, the frequent failure of this form to bear telia abundantly under conditions of heavy infection is caused primarily by its susceptibility to injury and defoliation from rust and drought before telia can be produced. The foregoing conclusions for this species, in addition to being supported by all results in the present tests, were further substantiated by observation of its behavior at two centers of heavy natural infection in north Idaho. The part-shade form generally resembles the shade form in its reactions.

The data for both forms of *Ribes lacustre* and *R. viscosissimum* in table 10, with the possible exception of the results at Camp McKinney for the shade and open forms of *R. lacustre* and the shade form of *R. viscosissimum*, indicate a high degree of variability in seasonal reaction to the rust within a relatively limited range of susceptibility. As a rule these ribes species show a considerable degree of variation in their group reaction from season to season both in percentage of leaf surface infected and percentage of infected surface bearing telia. In other words, they may vary from almost immune in an unfavorable season to moderately susceptible when conditions are especially favorable for rust development. Of the two, *R. viscosissimum* appears the more dangerous as a potential source of infection of white pines, at least as far as ability to produce telia is concerned.

Elevation is a local environmental factor which has an influence on the phenology of both the rust and the host. The short growing seasons at high elevations provide a more limited period for uredial intensification. At such elevations, however, the greater and more frequent precipitation may create more opportunities for infection, thus resulting in a greater abundance of rust on ribes at higher than at lower elevations, particularly in a dry season.

Another environmental factor of some local importance in variation in the development of rust is the occurrence of dew. At the Kelowna swamp and to a lesser degree at Canyon and Nine Mile Creeks there was evidence that heavy dews caused uredial intensification. This was also observed along the bottoms of many of the moist canyons in the white pine belt of north Idaho. Intensification from dews is generally much less than that caused by rains.

From the foregoing discussion it is evident that weather and local environmental conditions influence the development of the rust on ribes directly and by affecting the host. The important effect of host phenology upon initial infection and subsequent intensification has been mentioned. For the best initial infection it is essential that the period in which ribes leaves are in their most susceptible stages of development be synchronized with the period of maximum aeciospore production and with moisture conditions favorable for infection. To assure the maximum intensification thereafter, moist periods favorable for infection should come at frequent intervals coinciding with periods of abundant urediospore production. Normal irregularities in weather during the season almost invariably prevent any such ideal coincidence of favorable factors, although such conditions may occasionally be approached in one locality or another. In this respect climate is perhaps primarily important in its influence on the rust. On the other hand, abnormally cold or hot spells or other unseasonable weather conditions at any time during the growing season may and frequently do completely upset the processes of infection and inten-

sification, primarily through their influence on the host. Thus, to use an extreme example, the development of ribes leaves and aeciospores may be stimulated by early-season warm weather, rains may follow and good infection take place, and then the plants may be locally or generally defoliated by frost or drought.

To summarize, *Ribes petiolare*, disregarding its resistant form, which probably does not represent more than 10 to 15 percent of the species, and disregarding exceptional cases where a rather high degree of resistance was encountered, appears to be the most uniform of all species in its local and seasonal reaction to the rust. In general, it ranges from moderately to very highly susceptible and produces telia abundantly from the infected surface. The shade form of *R. inerme* exhibits similar uniformity but is less susceptible and considerably lower in telial production. The open form of this species shows considerable differences in amount of infection and telial production from one year to another. This variation is apparently caused by sensitiveness of this form to abnormal temperatures and drought. *R. lacustre* and *R. viscosissimum* are generally resistant, but in their more susceptible forms, and particularly in the case of *R. viscosissimum*, may be moderately susceptible when weather conditions are favorable.

GENERAL RESULTS

The results of the study indicate a susceptibility and capacity for telial production in *Ribes petiolare* approaching that of the cultivated black currant from plantings of which damaging infection has been known to spread to pines for over a mile. *R. inerme* compared favorably with other highly susceptible wild species from concentrations of which instances of similar spread have been observed. Even the relatively low telial production of *R. viscosissimum* and *R. lacustre* is known to be sufficient under moderately favorable conditions to seriously damage associated pines. The studies have given results of basic value in devising scouting programs to determine the extent of spread of the rust,[8] and in the development of control plans (*20*).

SUMMARY

This paper reports the results of studies of the white-pine blister rust in British Columbia and Idaho. The investigations were undertaken to determine the susceptibility and telium-producing capacity of the four principal ribes species in the commercial range of western white pine (northern Idaho, northeastern Washington, and western Montana), as a preliminary measure of the potential pine-infecting power of these species.

Since the tests were concerned entirely with the probable comparative importance of these ribes species in spreading infection to pines, methods used in other rust investigations were not applicable. New systems of recording data, developed to meet the needs of the study, are fully described.

The four species studied were *Ribes petiolare*, *R. inerme*, *R. viscosissimum*, and *R. lacustre*, all of which were present in the range of the rust in British Columbia. The studies were conducted under

[8] BLISTER RUST IN THE FAR WEST. Jan. 1 to Dec. 31, 1928. Spokane Branch Off. Blister Rust Control. 379 pp. [Typewritten report] See pp. 178–188.

natural conditions and included 5,098 tests (nearly 3,000,000 leaves on 2,740 different bushes) in interior British Columbia, from 1924 to 1928, inclusive, and 1,358 tests (nearly 500,000 leaves) in the coastal region of British Columbia. Results were substantiated by 562 tests (nearly 400,000 leaves) conducted in the white pine region of northern Idaho during 1933 and 1934. Ribes species other than those studied are seldom encountered in the commercial range of western white pine.

Ribes petiolare was found to be extremely susceptible, approaching cultivated black currant in both severity of infection and production of telia. *R. inerme* is also highly susceptible, under very favorable conditions, equaling or surpassing *R. petiolare* in extent of infection, and bears abundant telia when growing in the shade. *R. viscosissimum* and *R. lacustre* are more resistant. The latter occasionally becomes moderately infected, but is almost always quite low in production of telia. When density and size of telial columns are considered, the relatively greater teliospore production and potential danger to associated pines of *R. petiolare* and *R. inerme* appear even more pronounced. Infection is heavier and telia are more abundant on plants in the shade than in the open. Data on average infection, telium production, and teliospore production are given for open, shade, and part-shade forms of each species.

Relative susceptibility and ratios of telium production to percentages of leaf surface infected remained fairly constant, but weather and other environmental factors caused pronounced local and annual differences in the degree of infection between individuals and groups of individuals of the same species and form. It also appeared that variation was affected by the inherent differences in susceptibility between individuals.

Results of the studies have been of basic value in formulating plans for control of the rust in northern Idaho and adjacent Montana and Washington.

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A MICROCHEMICAL COLORIMETRIC pH PROCEDURE FOR DIFFERENTIATING THE TELIA OF CRONARTIUM RIBICOLA AND C. OCCIDENTALE[1]

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INTRODUCTION

Efficient control of the white-pine blister rust (*Cronartium ribicola* Fisch.) is dependent upon early recognition of advance infections on ribes. As the distance between the areas in the West infected respectively with *C. ribicola* and with *C. occidentale* Hedgc., Beth., and Hunt grows shorter, the differentiation of these two rusts in all stages becomes increasingly necessary. The present work was undertaken because there was no known method of differentiating the two rusts in the telial stage. Very frequently from August to the end of the season the ribes leaves collected bear telia only. It was highly desirable that the differentiation be a quick, 100-percent effective method, applicable to small and sparsely infected specimens such as advance infections are likely to furnish. All the standard biological stains that had been tried failed to give any differentiation. Measurements of length and width of the telia of the two species gave unsatisfactory data, showing definite differences between the averages of a considerable number of specimens, but overlapping and therefore of no significance when applied to one unknown sample.

It was assumed that certain constant differences in morphology, physiology, and behavior occurring in the other spore stages of these two species were concurrent with some basic physical-chemical difference in the telial stage, however small or variable. Because pH measuring methods indicate by color very minute differences in acidity or alkalinity, which can have marked influence on the physical-chemical properties and reactions of tissue constituents, a series of exploratory studies of pH reactions was made.

RESULTS OF EXPLORATORY EXPERIMENTS

Though the exploratory studies involved different methods of approach and were constantly refined and changed, from the very beginning all the findings were consistent and supported the theory of the reactions occurring in this procedure.

The results of these preliminary studies showed some chemical difference in the two species as indicated by their action upon various indicators, the effects of indicators on them, and differences in the pH values of measured quantities of water in which weighed amounts of telia were extracted. In each case, however, the differences were

[1] Received for publication Feb. 16, 1937; issued September 1937. This work, which was done at the Bureau of Standards, U. S. Department of Commerce, is one of a series of cooperative investigations carried on by that Bureau and the Division of Forest Pathology, Bureau of Plant Industry, U. S. Department of Agriculture. The research was supported in part by Emergency Conservation Work.

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those between the averages for a number of telia, and overlapped in much the same way as spore measurements. They were consistent for not over 70 percent of the specimens tested and negative or without significance for the others, or else very slight or transitory. For instance, some indicator solutions or mixtures containing indicator solutions produced colors in the two species differing in hue as much as two indicator solutions 0.1 to 0.5 pH apart. It was, however, impossible to establish color standards with any of the methods developed in these preliminary experiments because the initial color of the telia of both species is yellowish, ranging from light honey to reddish brown, and varies in hue and intensity more within a single species than an indicator varies in hue and intensity over 0.4 to 0.5 Hp. Furthermore the phenolsulfonphthalein indicators, the precise application of which to pH problems has been developed [3] [4] by S. F. Acree and his coworkers are yellow on the acid side and intensely red, green, or blue on the alkaline side of their range. Obviously the combination of the variable initial color of the telia with the color of the indicator results in color massing and proportionate variation in the final color, and only a procedure resulting in two distinct colors, separated on the spectrum, would supply unmistakable differentiation of these two species. As pointed out previously, it was also necessary to have a method applicable to both sparsely and heavily infected specimens.

EQUIPMENT AND PROCEDURE

A procedure was finally developed that caused the telia of *Cronartium occidentale* to appear a bright green and those of *C. ribicola* a brilliant blue, when viewed under the compound comparison microscope or the compound microscope with a magnification of 108 ×. Citations to color charts for a more accurate description of these colors are given along with other details of the procedure.

The procedure consists essentially in consecutive measured exposure of the telia to 0.1 N hydrochloric acid, distilled water, and 0.001 M bromphenol blue adjusted to pH 7.6 (three units above the pH color range (3.0–4.6) of this indicator). Exposure to the acid and water is measured by the time interval of and the amount of agitation during each step. Exposure to the indicator is controlled by regulating the thickness of the film of indicator in the mount. The exploratory experiments gave evidence of a minute but not easily demonstrated physical-chemical difference between the two species. The procedure intensifies and amplifies this difference and expresses it in two definite and unmistakable colors.

The critical factors in the procedure seem to be the balance between the exposure of the telia to the hydrochloric acid and to the water and the choice of indicator and its pH adjustment. If the concentration of the acid solution, the length of the time intervals in acid or water, or the pH value of the bromphenol blue are changed at random, the results vary over a wide range of yellow, brown, green, blue, purple, and mixtures of all or any of these colors, or the two species

[3] BIRGE, R. T., and ACREE, S. F. ON THE QUINONE PHENOLATE THEORY OF INDICATORS. A SPECTROPHOTOMETRIC METHOD FOR MEASURING THE CONCENTRATIONS OF QUINOIDAL AND LACTOIDAL SALTS AND THE EQUILIBRIUM AND AFFINITY CONSTANTS OF THE PHENOLPHTHALEINS AND PHENOLSULFONPHTHALEINS. Jour. Amer. Chem. Soc. 41: 1031–1050. 1919. (Contains references to earlier papers.)

[4] ACREE, S. F., and FAWCETT, E. H. THE PROBLEM OF DILUTION IN COLORIMETRIC H-ION-MEASUREMENTS. II—USE OF ISOHYDRIC INDICATORS AND SUPERPURE WATER FOR ACCURATE MEASUREMENT OF HYDROGEN-ION CONCENTRATIONS AND SALT ERRORS. Indus. and Engin. Chem. Analyt. Ed. 2: 78–85. illus. 1930. (Contains references to papers appearing after 1919.)

may stain the same shade of green or blue, different shades of green or blue, or both green and blue in the same mount. It is probable that variations in the exposures and concentrations used in this procedure could be rearranged so that the balance would not be destroyed and the results would be the same.

The success of this procedure indicates that a combination of microchemical technique and pH procedure may offer new possibilities for the differentiation by color of species and varieties otherwise indistinguishable. There is a definite need for such methods in the study of such groups of fungi as the rusts, where the lack of any known method of artificial cultivation deprives the investigator of this prolific source of criteria for species identification.

For readers who may be concerned with solving a similar problem, the procedure will be described as it was carried out during the development of the process and will, therefore, include the equipment and handling necessary for putting two preparations through the same treatment, separately and as nearly simultaneously as possible. Any discrepancies in equal processing of the two samples, due to the necessity of starting one first, were kept at a minimum by starting one sample first in each separate step of the process and then repeating and starting the other sample first each time.

The following items were assembled ready for use:

(1) A dissecting microscope; wide-angle lenses greatly increase comfort and efficiency.
(2) A comparison microscope equipped with a 9× ocular and 16-, 8-, 4-, and 2-mm objectives, but for these experiments the 16-mm objective would have been sufficient.
(3) Samples to be tested: Pieces of ribes leaves one-eighth to three-eighths of an inch across bearing some normal fully developed telia.
(4) A clean, highly polished curved scalpel with square end and convex cutting edge.
(5) A clean, polished, slender-tipped pair of forceps.
(6) A small water-color camel's-hair brush with the bristles cut off straight across the thickest part of the brush.
(7) A bottle of 0.1 N hydrochloric acid.
(8) A beaker of 100-cc capacity, containing 50 cc of distilled water.
(9) Four glass jars or flasks with tops or stoppers of about 30-cc capacity, each containing 25 cc of distilled water; two jars to be used for first washing and two for second washing.
(10) A supply of small torn pieces of absolutely clean dry filter paper.
(11) Some chemically clean cover glasses and slides, with labels. The labels must be pasted on without smearing excess glue out onto the slide, to avoid chemical contamination.
(12) Wash bottle or beaker containing distilled water.
(13) A waste jar or beaker.
(14) Two watch glasses about 2 inches in diameter, each containing 1 cc of 0.1 N hydrochloric acid. This acid should be put in the watch glasses just before starting the test.
(15) 0.001M bromphenol blue adjusted to pH 7.6, preferably in a pyrex dropping bottle.
(16) Some sterile surgeons' gauze.

To cut down the time of manipulation and so keep the processing of the samples of the two species as nearly simultaneous as possible, the same camel's-hair brush, forceps, and scalpel were used in each step and for both species. After each single manipulation, however, the instrument used was washed in distilled water and dried on sterile surgeon's gauze. The possibility of introducing error-producing chemicals through contact with the gauze was checked by previously boiling the gauze in distilled water for 10 minutes and comparing the

pH reading of this water with that of the same quantity of distilled water from the same source, boiled 10 minutes. The gauze produced no change in the pH value of the water.

The procedure is as follows:

(1) Note the starting time and drop the samples of leaves, with telia-bearing surfaces down, into watch glasses containing 2 cc of 0.1 N hydrochloric acid.

(2) Under the dissecting microscope, reverse the samples so that the telia are up and with the camel's-hair brush tease air bubbles from the telia and leaf surfaces; turn back so that the telia-bearing surfaces are down; agitate by giving the watch glasses two left-to-right motions with the hand.

(3) Twenty minutes after starting time, agitate again by two left-to-right motions with the hand.

(4) Twenty-seven minutes after starting time, agitate again.

(5) Thirty minutes after starting time, with forceps remove the leaf samples from the acid, touch with filter paper, pass through the water in the 100-cc beaker, touch with filter paper to remove excess water, and put into the jars containing the first wash water and agitate.

(6) Forty-five minutes after starting time, agitate the jars containing the samples and first wash waters, and with forceps transfer the samples to the second wash water and agitate.

(7) Sixty minutes after starting time, agitate the second wash waters containing the samples, remove the samples with forceps to the slides and place them to one side of mounting spaces on slides that are already in focus under the dissecting microscope.

(8) Draw excess water from the samples with filter paper but leave enough to keep the telia thoroughly wet.

(9) Putting the scalpel close to the bases of the telia, lift or drag them from the leaf. When about a dozen are removed to each slide drag them to the centers of the areas intended for mounts, remove excess water with filter paper, put enough bromphenol blue, pH 7.6, on each lot of telia to make a film between the slide and cover glass about the same thickness as the thickness of the telia, and put on the cover glasses.

(10) Remove the slides at once to the comparison microscope for observation. During the process of removing the telia from the leaf samples and putting on the bromphenol blue, pH 7.6, the telia must stay wet, but excess pools of water must be avoided. Obviously an excess of water would affect the indicator. Under the comparison microscope it will be observed that the indicator immediately surrounding the telia of both species at first turns yellow. This yellow slowly diffuses into the indicator of the remainder of the mount, and more or less rapidly the telia of *Cronartium ribicola* become blue and those of *C. occidentale* green. This takes from 5 to 15 minutes.

(11) When the blue or green of the telia approaches its maximum brilliancy, place the slides under the dissecting microscope and with filter paper draw off all the indicator possible. This must be done quickly and be so manipulated that all is drawn from around the telia at least, so that any small remaining amount must not contact the telia. Press the cover glass firmly down upon the telia and slide by applying weights or wooden pinch-type clothespins. This must be done so that the mount is as nearly as possible the thickness of the telia but mashing of the latter should be avoided. A little experience

is needed to develop judgment as to when to draw off the indicator to secure brilliant but not overstained preparations and how much pressure will make a thin mount without crushing the telia.

Staining with the residual indicator is evidently greatly intensified by the evaporation of the water and proportionate increase in the concentration of the indicator. This can be easily and quickly controlled by running paraffin oil under the cover glass as soon as the colors attain the desired intensity. This makes a clearer, more transparent preparation, but the mount is too messy and dust-collecting for anything but temporary use.

Understaining results in softer, less true blues and greens; overstaining, in bluish or greenish black. The mashed telia are always paler and sometimes a less true blue or green. This may be due to the smaller mass of color in a thinner layer of cells or to some chemical reaction that occurs when the tissue is crushed. It is not necessary to leave the clothespins on more than 20 to 30 minutes, but no harm is done if they remain on overnight. After the clothespins or weights are removed, these preparations can be stored away from the dust and kept for several months without ringing with balsam, but it is necessary to handle them carefully to prevent the cover glass from slipping off. For this reason slides that are to be kept should be ringed with Canada balsam or collodion too thick to run under the cover glass. To facilitate ringing, the balsam can be warmed; but if it contacts the telia, their color is sometimes changed. Preparations sealed with balsam or collodion retain their transparency and full differential color values for at least a year.

Oil mounts can also be made by removing the clothespins or weights and the cover glass, adding a drop of paraffin oil, replacing the cover glass and ringing the mount with Shuh's cement. Very clear slides that will keep from 6 to 10 weeks are so obtained. In time, however, the colors lose their brilliancy.

As stated previously, the differential characteristic of this process is that *Cronartium ribicola* stains blue and *C. occidentale* green. The blue will vary from a brilliant deep hue to a duller or paler blue and the green from a deep bluish green to a softer grayer or pale green. Not all the telia on a slide stain evenly; some are deeper or paler, harder or softer, more or less true blue or green, but *C. ribicola* appears definitely blue and *C. occidentale* definitely green, under a magnification of 108 ×. For accurate description of these colors, Ridgway's [5] Color Standards and Maerz and Paul's [6] Dictionary of Color were consulted. Because of the transparent quality of the colors in the latter, both the greens and blues were best matched in it. The blues corresponding most closely to the blues in the *C. ribicola* telia are found in Maerz and Paul's plate 34 L 7–12, and the greens matching those in *C. occidentale* best are found in their plate 30 L 6–12. Of Ridgway's colors, the blues matching best are those in plate VIII, light cerulean blue, cerulean blue, oxide, and Antwerp blue, and the greens matching best are those in plate VII, light blue, Guinea, and dark viridian green, and plate XVIII, oriental and dark yellowish green.

Upon further study with a magnification of 160 × or more, it is evident that the blue telia of *Cronartium ribicola* are made up of

[5] RIDGWAY, R. COLOR STANDARDS AND COLOR NOMENCLATURE. 43 pp., illus. Washington, D. C. 1912.
[6] MAERZ, A., and PAUL, M. R. A DICTIONARY OF COLOR. 207 pp., illus. New York. 1930.

white matrix or cell wall and deep-blue spores or spore contents with sometimes a little very pale yellow matrix at the tip. Those of *C. occidentale* have a yellow or pale yellow-green matrix, green or green-blue spores or spore content, with sometimes a few scattered patches of blue where the indicator has evaporated on the telia surfaces. The blue when present is not the clear, brilliant, and undiluted blue of the *C. ribicola* telia, but dull and greenish. The bright-green color seen under the lesser magnification is apparently the composite color of the layers of pale and deep green, yellow and green, yellow and blue, green or green blue. For differentiation purposes a magnification of 108 × or less is best, because it shows clearly and definitely blue telia if the specimen is *C. ribicola* and green if it is *C. occidentale*. The reliability of this procedure for identification was tested on 173 specimens of known origin.

The development of this procedure involved a great deal of experimentation in precise technique which was greatly facilitated by the use of the dissecting and comparison microscopes. The dissecting microscope assists in the elimination of such sources of inaccuracy as air bubbles on the telia and leaf surfaces in the acid bath, and excess water in the mount. The simultaneous observation of the color changes in the two mounts, made possible by the use of the comparison microscope, saves innumerable timing and time records, and obviates the necessity of depending upon the memory for color comparisons.

SUMMARY

The established methods of species identification failed to furnish means of differentiating *Cronartium ribicola* and *C. occidentale* in the telial stage.

It was assumed that certain constant differences in the morphology, physiology, and behavior occurring in the other spore stages would be concurrent with some slight but basic physical-chemical difference in these two species that would occur also in the telial stage. Studies involving pH reactions were made because of the minute differences that such methods reveal and with the hope that if such a difference existed it could be demonstrated unmistakably by color.

Many exploratory experiments showed that there was a definite and consistent but not easily demonstrated microchemical difference between the two species. The objective then became the development of a treatment to amplify this difference and an indicator solution to demonstrate it. This was accomplished by certain treatments with dilute acid, distilled water, and bromphenol blue under pH control.

The results of this work indicate the opening of a new field of study for the solution of similar problems. The value of applying pH methods to problems involving study of very subtle biological differences lies in the delicacy and precision of the reactions. This is evidenced by the consistency of the results when different indicators were used in various ways and by the variable results caused by slight alterations in the procedure.

MERMIS SUBNIGRESCENS, A NEMATODE PARASITE OF GRASSHOPPERS[1]

By J. R. CHRISTIE[2]

Nematologist, Division of Nematology, Bureau of Plant Industry, United States Department of Agriculture

INTRODUCTION

In a previous paper[3] the author pointed out that in the United States the most common nematode parasites of grasshoppers are *Agamermis decaudata* Cobb, Steiner, and Christie, 1923, and *Mermis subnigrescens* Cobb, 1926,[4] species belonging to the family Mermithidae. The life history and the economic significance of the former species were discussed. The present paper deals with *M. subnigrescens*.

Whereas *Agamermis decaudata* is occasionally found in insects other than grasshoppers, *Mermis subnigrescens* appears to be strictly a parasite of grasshoppers. It has been found naturally infesting nine different species of these insects, including both Tettigoniidae and Acrididae; two additional species have been experimentally infested. It has not been found in crickets, even though they were collected in fields where a large percentage of the grasshoppers harbored the parasite. Numerous attempts to experimentally infest other insects, including crickets and mole crickets (Gryllotalpinae), have not been successful.

Mermis subnigrescens occurs throughout the New England States and westward to Minnesota, Iowa, and Missouri. Its range apparently does not extend as far south as that of *Agamermis decaudata*, for it is rarely found in the vicinity of Washington, D. C. The present paper is based on investigations conducted largely at Woods Hole, Mass., and statements regarding life history and behavior apply to that locality.

LIFE CYCLE

The life cycle of *Mermis subnigrescens* differs in one important respect from that of *Agamermis decaudata*. Eggs of the latter species are deposited in the soil. Upon hatching, the larvae migrate to the surface, climb the vegetation, and seek newly hatched grasshopper nymphs, which they enter by boring through the body wall. Eggs of *M. subnigrescens* are never deposited in the soil. Gravid females migrate to the surface and climb the vegetation, on which they deposit their eggs. Grasshoppers, while feeding, swallow the eggs, which on reaching the alimentary tract promptly hatch. The larvae penetrate the wall of the alimentary tract and enter the body cavity, where they develop.

[1] Received for publication Feb. 16, 1937; issued September 1937.

[2] The author wishes to acknowledge the aid of W. D. Courtney, who assisted with field work during the summer of 1929; of B. G. Chitwood, who assisted during the summer of 1930; and of Gerald Thorne, who assisted during the summer of 1931. Figures 1, *B*, and 3 were taken from unpublished notes of the late N. A. Cobb.

[3] CHRISTIE, J. R. LIFE HISTORY OF AGAMERMIS DECAUDATA, A NEMATODE PARASITE OF GRASSHOPPERS AND OTHER INSECTS. Jour. Agr. Research 52: 161–198, illus. 1936.

[4] COBB, N. A. THE SPECIES OF MERMIS, A GROUP OF VERY REMARKABLE NEMAS INFESTING INSECTS. Jour. Parasitol. 13: [66]–72, illus. 1926.

THE EGG

The egg (figs. 1, *A*, and 2) is protected by two coverings, the outer of which is easily removed. With this outer covering removed, the egg (fig. 1, *B*) has the shape of a sphere slightly compressed at the poles.

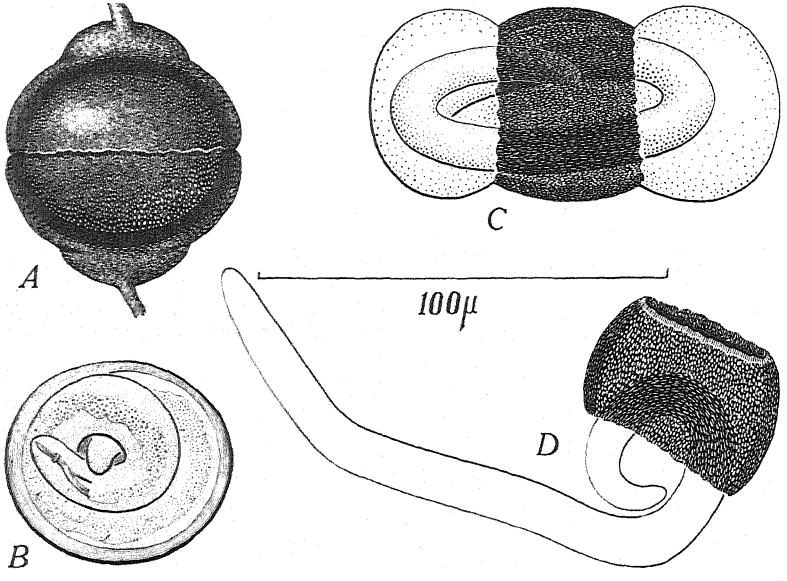
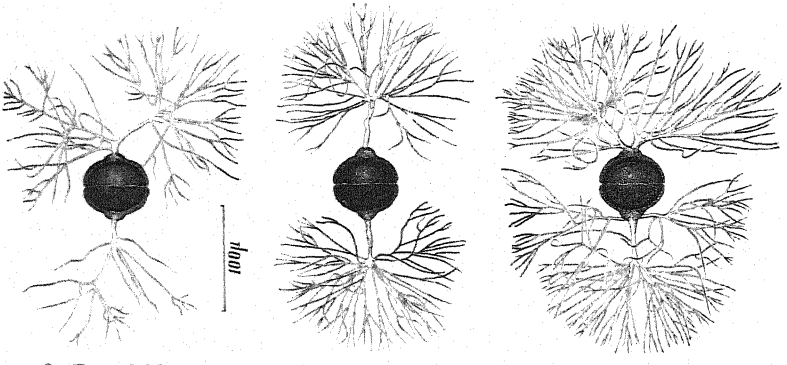


FIGURE 1.—Egg of *Mermis subnigrescens*: *A*, Showing outer and inner layers of shell; *B*, with outer layer of shell removed, showing larva within; *C*, in process of hatching; *D*, with larva emerging.

The diameter from pole to pole is 50μ to 54μ and at the equator 53μ to 56μ. The inner covering or shell is about 2.5μ in thickness and brown in color. The outer covering is divided into two cuplike



URE 2.—Eggs of *Mermis subnigrescens* showing variations in the form of the byssus. (Drawn from photomicrographs.)

parts by a groove at the equator, and at each pole there is a raised or thickened area formed by the attachment of the entangling appendages or byssi. The size and shape of these polar thickenings vary considerably, as does also the form of the byssus (fig. 2). There are

two opposite areas at the equator where the color is lighter than elsewhere, and these areas are partially dissolved by the digestive fluids of the host, permitting the escape of the larva. At the time of deposition the egg contains an infective larva that has molted at least once (fig. 1, *B*).

The time of maximum egg deposition is usually during June and July, but, as will be discussed later, this is controlled by weather conditions. On foliage an egg remains viable throughout the summer, the larva presumably being protected from the sun's rays by the brown pigment in the shell. Eggs kept in water in a corked bottle at room temperature remain viable for at least 2 months; when kept on slightly moistened filter paper in a moist chamber some remain viable for a year.

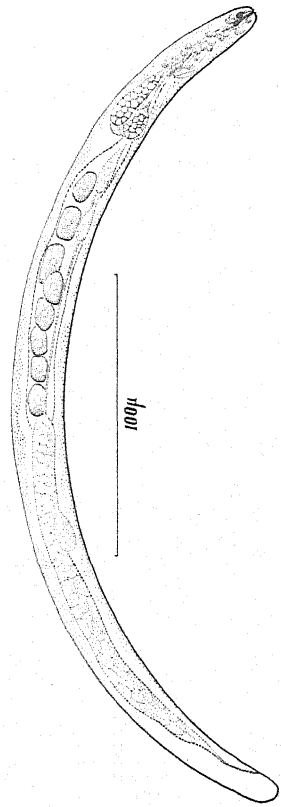


FIGURE 3.—Recently hatched larva of *Mermis subnigrescens*.

MODE OF INFESTATION

Grasshoppers become infested, while feeding, by swallowing eggs of the parasite, and therefore they are vulnerable throughout their entire life. As a nymph grows older and its food consumption increases, its chance of becoming infested is correspondingly greater. The fact that a nymph becomes infested while young does not prevent it from acquiring additional parasites later in life. Grasshoppers are frequently found that harbor a hundred or more parasites of widely different ages.

When an egg reaches the alimentary tract the outer covering bearing the byssi has usually been rubbed off. The two opposite clear areas at the equator gradually become clearer and begin to protrude until they appear as colorless, hemispherical projections (fig. 1, *C*), which finally rupture and provide an opening for the escape of the larva (fig. 1, *D*). So far as has been determined, the larva in no way assists in its liberation. When first freed it is rather sluggish, although it quickly becomes active and soon penetrates the wall of the alimentary tract and enters the body cavity. This migration is discussed more fully in a later section.

The recently hatched larva (fig. 3) is about 740μ long by 34μ wide. A stylet of peculiar form is present, which can best be understood by reference to figure 1, *B*. The esophageal region, which comprises nearly half the length of the body, contains eight large esophageal glands (stichocytes) closely resembling those of *Agamermis decaudata*. The body ends posteriorly in a rounded terminus. A node and a postnodal region are lacking.

PARASITIC LAVAL STAGE

The parasitic development of the larva has not been carefully studied, but it seems essentially the same as that of *Agamermis*

decaudata. There is the same rapid increase in size and the same type of trophosome development.

Males usually remain in the host from 4 to 6 weeks and attain a length of from 20 to 60 mm. Females usually remain in the host from 8 to 10 weeks and attain a length of 50 to 160 mm. However, both the duration of the parasitic stage and the size of the fully grown larva are subject to great variation. In general the fewer parasites the insect harbors, the larger they become and the longer they remain in the host. Likewise there is a tendency for these parasites to grow larger and remain longer in a large grasshopper than in a small one.

The emergence of *Mermis subnigrescens* results in the death of the host. A grasshopper may harbor parasites of widely different ages, and when the oldest ones emerge most of the others perish with the host. However those that are almost fully grown may also emerge and enter the soil. The same thing happens when a parasitized grasshopper dies from some other cause: the parasites that are nearly full grown may emerge, the others will die. Many of these prematurely emerged individuals eventually die in the soil, but some undoubtedly survive. The parasites begin emerging at least by the first of July and continue throughout the summer as long as grasshoppers are alive. The emergence of *M. subnigrescens* from its host is similar to that described for *Agamermis decaudata*.[5]

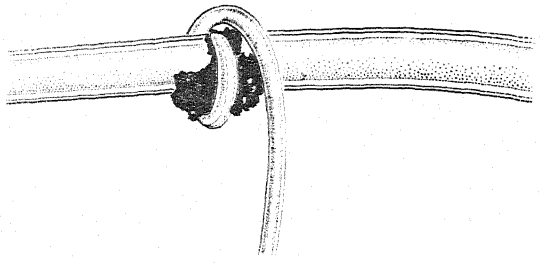


FIGURE 4.—Copulation in *Mermis subnigrescens*. (Freehand sketch.)

The final molt occurs the following spring. Experimental specimens buried at Woods Hole during the autumn of 1927 had molted or were in the process of molting when examined on April 12, 1928.

ADULT STAGE

Mermis subnigrescens is found in the soil at various depths down to about 24 inches. The majority occur from 6 to 18 inches below the surface. As a rule, individuals remain alone and one rarely finds a female and one or more males coiled into a knot as is characteristic of *Agamermis decaudata*. Copulation takes place at least occasionally and has been observed several times (fig. 4). By July females that emerged from their hosts the preceding summer begin to exhibit a brownish color owing to accumulating eggs, and by September they appear nearly black except for a short region at each extremity of the body. At this time the eggs are viable.

Oviposition takes place the following spring, beginning about the first of June and continuing until the first of August or possibly longer. In 1927 eggs were first found on the foliage May 27, but it was not until June 6 that they were numerous; in 1929 they were first found June 1, and by June 25 and thereafter they were numerous. Eggs are laid only during rains, and should there be no rain in June they will not be deposited in any number until rain occurs.

[5] CHRISTIE, J. R. See p. 187 of citation given in footnote 3.

Eggs are apparently not deposited during the night, a point that is discussed in a later section. At daybreak on a rainy morning in June or July ovipositing females are found moving about on the grass and other vegetation. If the rain continues, egg deposition goes on throughout the day, but if the rain ceases and the foliage becomes dry, the females coil up, fall to the surface of the ground, and enter the soil. Should the supply of eggs not be exhausted, a female presumably comes to the surface and resumes egg laying during a subsequent rain; however, the author was not able to demonstrate this experimentally. Before ovipositing, a gravid female 85 mm long contains approximately 14,000 eggs.

When laying eggs a female is entwined about a grass stem or other object (fig. 5), and the head end is extended farther and farther from

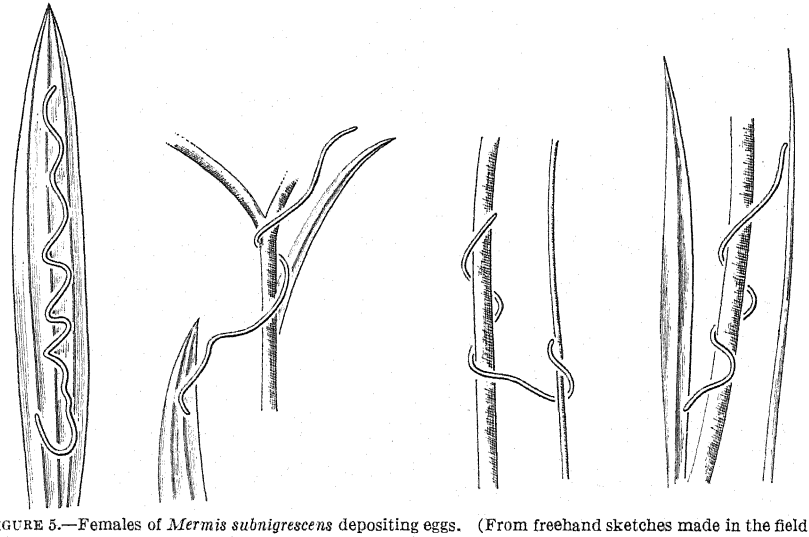


FIGURE 5.—Females of *Mermis subnigrescens* depositing eggs. (From freehand sketches made in the field.)

the supporting stem and is moved about with a more or less circular motion. If another stem is nearby, the anterior end is entwined at a point somewhat higher than the mermithid's position on the first stem. Thus, in dense vegetation a female, as it moves horizontally, is likely to mount higher and higher. However, if no object is nearby, more and more of the anterior end of the body extends until it begins to sag, and another or even the same stem may be entwined at a point nearer the ground.

How long a female lives after the uteri are emptied of eggs has not been determined. Since by this time the trophosome is nearly exhausted of reserve food materials, it seems unlikely that the female is able to survive another winter or to develop more eggs. However, if females are prevented from coming to the surface to deposit eggs, they will survive for several years.

Some of the males and females that emerged from hosts during September 1930, and were buried in containers at Woods Hole on September 25, 1930, were alive and in apparently good condition when examined on May 15, 1933. The females were filled with eggs, and there was no indication that any considerable number had been

deposited. Normally these individuals would have laid their eggs during June and July 1932, but being in containers they were unable to come to the surface. Sunlight is apparently the stimulus that causes egg laying. If an ovipositing female is placed in the dark, egg laying immediately stops; if again it is brought into the light, egg laying is promptly resumed. This interesting behavior has been described by Cobb.[6]

SECURING MATERIAL FOR STUDY

A limited number of postparasitic specimens of *Mermis subnigrescens* can usually be secured by digging, but this is a slow and laborious procedure. During the course of these investigations considerable digging was done not primarily for the purpose of securing material but to determine the abundance and distribution of the parasites in the soil. When securing material is the object, the operation can be facilitated by the use of sieves.

Adult females of *Mermis subnigrescens* can often be collected in large numbers while they are laying eggs. The collector should know the location of fields where these mermithids occur, and the fields must be visited during rainy days at the proper season of the year. On June 8, 1930, at Woods Hole, during a period of about 3 hours, the author collected 220 ovipositing females.

Probably the most practical method of obtaining *Mermis subnigrescens* in large numbers is to collect infested grasshoppers and confine them in cages until the parasites emerge. For this purpose any type of insect cage will serve if so constructed that 2 or 3 inches of soil can be placed in the bottom. In this soil the author usually planted wheat, which was allowed to grow a few inches high before the grasshoppers were placed in the cage. When one has less access to a field where there is a fairly high infestation a great many parasites can be secured in this manner. When grasshoppers are collected in fields and confined in cages a considerable number usually die. The death rate is highest during the first day or two and then gradually decreases. The result is many prematurely emerged parasites, which is the chief disadvantage of this method of securing material. It can be overcome to some extent by allowing the nematodes to remain in the soil of the cage for several weeks. During this time many of the prematurely emerged specimens die and are eliminated. When subsequently examined, additional abnormal specimens usually can be recognized and discarded. The soil in the cage must be kept moist at all times.

The specimens of *Mermis subnigrescens* thus obtained can be buried in the soil, where they proceed with their development and where they can be dug up and examined from time to time. One of the best containers for this purpose is an unglazed porcelain battery jar with the open end closed by 200-mesh, nickel alloy, wire cloth. Small jars for individual specimens can be obtained, or large ones that will accommodate 100 specimens or more. The soil used to fill the containers and in which the mermithids are placed should be composed in part of sand and should be passed through a sieve. When the container is subsequently dug up the mermithids may be secured by again passing the soil through a sieve. If the soil is

[6] COBB, N. A. THE CHROMATROPISM OF MERMIS SUBNIGRESCENS, A NEMIC PARASITE OF GRASSHOPPERS. Jour. Wash. Acad. Sci. 19: 159–166, illus. 1929.

partly sand and has previously been sieved this can be done with little danger of injury to the specimens.

For concentrating these parasites in a small area, outdoor enclosures were constructed, 4 to 6 feet wide by 8 to 12 feet long, with the sides and top covered with ordinary window screening. Grasshoppers collected in fields of high infestation were placed in these enclosures, which were so situated that a naturally luxuriant vegetation served as food for the insects. The mermithid population of the underlying soil was undoubtedly increased in this manner, but even here the securing of specimens by digging was slow and laborious.

If an adequate source of infested grasshoppers is not available, uninfested grasshoppers may be collected and artificially infested. Such a method could be employed should an attempt ever be made to colonize the parasite in regions where it does not exist. The following procedure has proved fairly satisfactory. Leaves of the buckhorn plantain (*Plantago lanceolata* L.) were secured, and on them eggs of the parasite were sprayed by means of a small atomizer. The stems of the plantain leaves were then inserted into a small-mouthed vessel containing water, in the same manner as flowers are placed in a vase, and transferred to an insect cage. Any convenient cage can be used, the size depending on the number of grasshoppers to be handled.

The chief difficulty with this and other methods employed for artificially infesting grasshoppers is to prevent too many eggs from being swallowed. So many mermithids per host are acquired that most of the parasites develop into males.[7] Grasshoppers infested by this method must be held in confinement and fed throughout the entire developmental period of their parasites, while in those already infested when collected many of the parasites may be almost ready to emerge.

TIME OF EGG DEPOSITION

Rain stimulates females of *Mermis subnigrescens* to ascend vegetation and deposit eggs, a fact which was clearly demonstrated at Woods Hole in 1929. The spring and early summer were exceptionally dry, and very little rain fell between June 1 and July 19. On a plot of land where *M. subnigrescens* was known to be abundant, only a few eggs had been found on the foliage. On July 17, a lawn sprinkler was operated from 4:30 p. m. until 8 p. m. During this period of 3½ hours an average of about 2 inches of water was applied over an area approximately 14 feet in diameter. Early the following morning 12 mermithids were seen depositing eggs on the vegetation in this area, and undoubtedly there were others that were overlooked. None were found outside the sprinkled area. On the following day the sprinkler was moved to a new position, and the operation was repeated with similar results. In both sprinkled areas a subsequent examination of the foliage showed that eggs were present in abundance.

It has already been mentioned that light is apparently the stimulus that causes egg laying, a fact which indicates that oviposition cannot take place at night. On July 19, 1929, four ovipositing females were kept under constant observation from 7:30 p. m. until 9:45 p. m.

[7] CHRISTIE, J. R. SOME OBSERVATIONS ON SEX IN THE MERMITHIDAE. Jour. Expt. Zool. 53: 59–76 illus. 1929.

During this time rain fell intermittently. When it became so dark as to make observation impossible a pocket flashlight was used occasionally. By 8:50 it was nearly dark, and by 9:20 it seemed totally dark. Throughout early twilight all four females continued their characteristic movements, but as darkness increased they became gradually less active. At 9:40, about 30 or 40 minutes after the last vestige of daylight had apparently disappeared, two of the females were still on the vegetation but had ceased moving about. The other two had fallen to the surface of the ground, where they remained partly coiled up and comparatively inactive. Not one appeared to make any attempt to enter the soil.

At intervals one of the females was removed from the vegetation and placed on a wet leaf, where it was allowed to remain for half a minute or a minute (table 1). The leaves were later examined and the eggs counted. The results appear to indicate further that eggs are not laid at night.

TABLE 1.—*Effect of darkness on rate of egg deposition by Mermis subnigrescens*

Time of day (p. m.) female was placed on leaf	Period left on leaf	Light conditions	Eggs found on leaf
			Number
7:45	½ minute	Early twilight	21
8:20	½ minute	Late twilight	18
8:50	½ minute	Almost dark	41
9:20	1 minute	Dark	41
9:40	1 minute	do	0

DISSEMINATION OF THE EGGS

Eggs of *Mermis subnigrescens* were found on any object over which the ovipositing female had passed, whether living foliage or dead branches. There was no evidence that females selected any particular kind of foliage on which to oviposit. Eggs were found on either side of a leaf, sometimes in masses of 25 or more and at other times widely scattered. They were largely confined to herbaceous vegetation to a height of about 2 feet. On a few occasions they were found on the leaves of shrubs about 3 feet from the ground. The author saw no indication of dissemination by wind.

To facilitate the examination of a large amount of vegetation for the presence of eggs, the following method was employed: A piece of corrugated glass about 3 inches wide by 8 inches long, with the corrugations running lengthwise, was held on an improvised stand, in an oblique position, high enough from the table to allow a beaker to be placed under the lower end. A second beaker, containing 20 to 30 cc of water, was at hand. A leaf from the sample of foliage being examined was held on the corrugated glass with the left hand. A camel's-hair brush, with the bristles cut off to about one-third their original length, was repeatedly dipped into the water and passed several times over both surfaces of the leaf. Thus the eggs were dislodged and carried by the water down the corrugations of the glass into the beaker below. When the sample of vegetation was finished the remaining water was poured over the corrugated glass to wash any adhering eggs into the beaker. The water was then examined for eggs, usually after having been reduced in bulk by centrifuging. This method served well to demonstrate the presence or absence of

eggs, and when eggs were present it indicated with fair accuracy their relative abundance.

During the summer of 1929 an intensive study was made of egg dissemination in the vicinity of Woods Hole and in some of the adjoining localities of southeastern Massachusetts. The data secured are not given in detail. Suffice it to say that in fields where the parasite was present eggs were very widely disseminated. Samples of vegetation taken from the roadside, from back yards, and from other apparently unpromising locations were found very frequently to carry a few eggs.

To determine the vertical distribution of eggs a sample of grass consisting of all that grew on 1 square foot was collected during July 1929. Each stalk was cut close to the ground, the butts were placed evenly together, and the whole was cut into 2-inch lengths. Each 2-inch length was treated as a separate sample; every piece of grass in it, whether leaf or stem, was washed, and the washings were examined for eggs. The results are shown in figure 6.

On July 12, 1929, collections of grass and plantain leaves were made in 11 fields between Pawtucket, R. I., and Franklin, Mass. Eggs were found in all except two collections. That one could go into a region where data were not available regarding the distribution of this nematode, take 11 samples of vegetation at random from widely separated fields, and find eggs on every sample except 2 is further evidence that the parasite is widely distributed.

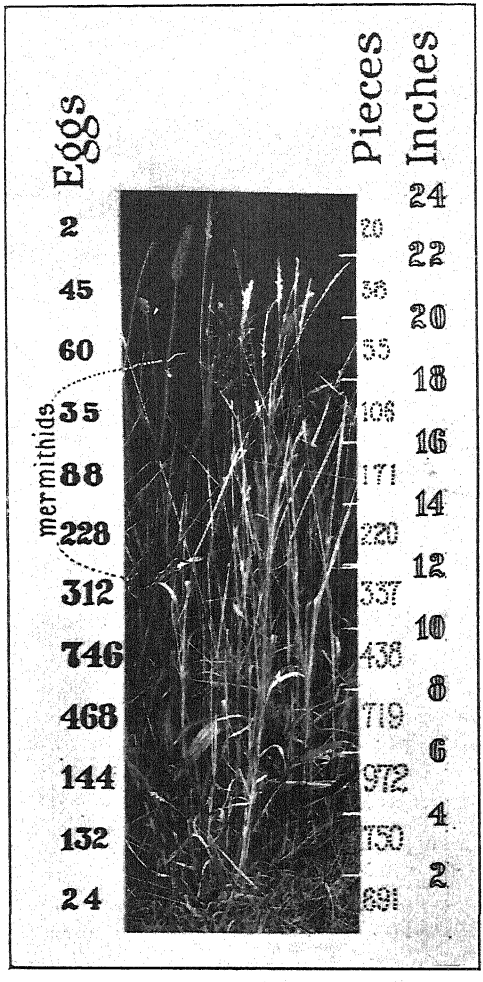


FIGURE 6.—Vertical distribution of eggs of *Mermis subnigrescens* on grass. The number of pieces of vegetation in each sample, the number of eggs found, and the height of each sample from the ground are indicated.

MIGRATION OF LARVAE FROM INTESTINE TO BODY CAVITY OF HOST

The hatching of *Mermis subnigrescens* eggs in the intestine of grasshoppers and the passage of the larvae through the intestinal wall into the body cavity were observed on several occasions. In making these

observations the following procedure was followed: Recently hatched nymphs of *Melanoplus femur-rubrum* (DeGeer) were placed in a cage and kept without food for 24 hours. A mash was prepared by mixing cracker crumbs, cane sugar, and orange juice. About 100 viable eggs were mixed with a bit of this mash to form a pellet a millimeter or so in diameter. This pellet and a grasshopper nymph were placed in a short glass tube corked at both ends. The nymph, when brought close to the pellet by patiently manipulating the tube, would usually eat. After a period of time varying from 30 minutes to 2 hours the nymph was killed, and its alimentary tract was removed and mounted on a slide under a cover glass in a drop of body-cavity fluid. A supply of adult grasshoppers was kept available to provide body-cavity fluid for this purpose. The preparation was then placed on the stage of a microscope for observation.

The first indication of hatching usually occurred after 1 to 1½ hours, when the eggs began to show the characteristic clear, hemispherical projections. Very soon after this the mermithid larvae were free in the intestine. At the end of 10 hours eggshells usually appeared in the feces. Most eggs hatched somewhere between the posterior end of the crop and the region where the Malpighian tubes are attached.

When first free from the shell the larva was comparatively inactive but soon began to move about. Sometimes 20 to 30 minutes elapsed before it passed into the body cavity. While penetrating the tissues of the intestinal wall the stylet was distinctly seen being rhythmically protruded. Most frequently larvae penetrated the intestine at the region immediately posterior to the caeca. On one occasion a larva entered a caecum and passed through its wall into the body cavity

EFFECT OF THE PARASITE ON THE HOST

The effect of *Agamermis decaudata* on the development of its host has been discussed in a previous paper.[8] In general the facts stated therein also apply to *Mermis subnigrescens*. The gonads of female grasshoppers are markedly inhibited in their development, and infested individuals are usually sterile. The effect on the development of the male gonads is less pronounced. In both sexes growth is materially retarded, infested individuals remaining in the nymphal stage longer than uninfested ones. A grasshopper is always killed when a parasite emerges. There is a tendency for the host to be older when it becomes infested than in the case of *A. decaudata*, and also for a host to harbor a greater number of parasites.

As already noted, *Mermis subnigrescens* apparently infests only grasshoppers. The following species were found naturally infested or were experimentally infested in the laboratory:

Species:	*Infestation*
Arphia sulphurea (Fabricius)	Experimental.
Camnula pellucida (Scudder)	Natural and experimental.
Chorthippus longicornis (Latreille)	Do.
Chortophaga viridifasciata (DeGeer)	Natural.
Conocephalus brevipennis (Scudder)	Do.
Encoptolophus sordidus (Burmeister)	Do.
Melanoplus bivittatus (Say)	Do.
M. femur-rubrum (DeGeer)	Natural and experimental.
M. mexicanus (Saussure)	Natural.
Orphulella pelidna (Burmeister)	Do.
Romalea micropiera (Beauvois)	Experimental.

[8] CHRISTIE, J. R. See pp. 186–189 of citation given in footnote 3.

DISTRIBUTION IN NEW ENGLAND

Between August 18 and 21, 1930, Gerald Thorne and the author collected and examined grasshoppers along the Merrimack River Valley between Manchester, N. H., and Woodstock, N. H., along the Connecticut River Valley between Middletown, Conn., and Woodsville, N. H., and from several localities in the interlying country. Collections were made in 83 places, and the number of grasshoppers examined in each collection varied from 10 to 100, depending on their abundance. The results may be summarized as follows:

Collections of grasshoppers:	*Grasshoppers infested (percent)*
10	None.
30	1 to 9.
19	10 to 19.
11	20 to 29.
7	30 to 39.
3	40 to 49.
2	50 to 59.
1	80.

A total of 2,500 grasshoppers examined showed an average infestation of 12 percent. The grasshoppers were not identified, and the collections were made up of various species, with *Melanoplus femur-rubrum* usually predominating. The mermithids from 66 of the collections were identified by Thorne, to whom the author is indebted for this information. In 42 collections the parasites were exclusively *Mermis subnigrescens;* in 17 collections both *M. subnigrescens* and *Agamermis decaudata* were present; in 6 collections the parasites were all *A. decaudata;* and in one collection *M. subnigrescens, A. decaudata,* and a species of *Hexamermis* were present.

About the same conditions exist in Barnstable, Bristol, and Plymouth Counties, Mass., where the author has made many collections of grasshoppers. *Mermis subnigrescens* is the most common mermithid parasite of grasshoppers in New England, and in this region it is very widespread. There are few fields where its presence cannot be demonstrated.

ECONOMIC SIGNIFICANCE

There is little doubt that both *Mermis subnigrescens* and *Agamermis decaudata* are important factors in grasshopper control throughout the regions where these parasites occur. Of the two, *M. subnigrescens* is perhaps the more important. It appears to be able to withstand a greater variety of soil and climatic conditions and to maintain itself in larger numbers where the grasshopper population is consistently low. It does not extend as far south as does *A. decaudata.* Insufficient rainfall has undoubtedly prevented the spread of both species into semiarid regions. Throughout much of the area in the United States where they are not found their introduction is evidently out of the question. Nevertheless there may be isolated regions where they do not exist but where climatic conditions are such that they could be established. Of the two species, *M. subnigrescens* is probably the best suited for colonizing. Its eggs are easily secured in large numbers, remain viable for months, and can be shipped any distance. The artificial infestation of indigenous grasshoppers should be a comparatively simple matter.

SUMMARY

Mermis subnigrescens Cobb, a common parasite of grasshoppers, occurs throughout the northeastern part of the United States and westward as far as Minnesota, Iowa, and Missouri. The present paper is based on studies made at Woods Hole, Mass.

The adults of this parasite occur in the soil. On rainy days during June and July gravid females come to the surface of the ground, ascend low herbaceous vegetation, and deposit their eggs. The eggs adhere to foliage by means of the entangling appendages or byssi. Grasshoppers, while feeding, swallow the eggs, which hatch in the alimentary tract. The larvae immediately migrate through the wall of the alimentary tract into the body cavity. They remain in the host from 4 to 10 weeks to complete their growth; then they emerge by forcing their way through the body wall and enter the soil. They molt the next spring, pass the ensuing summer and winter in the soil, and the females deposit their eggs during the following summer.

THE PRODUCTION OF CITRUS MOTTLE-LEAF IN CONTROLLED NUTRIENT CULTURES [1]

By H. D. CHAPMAN and A. P. VANSELOW, *assistant chemists*, and GEORGE F. LIEBIG, JR., *associate in the experiment station, California Agricultural Experiment Station*

INTRODUCTION

Although it is now definitely established that mottle-leaf of citrus can be controlled by the use of zinc sprays (*5*, *7*),[2] little is known about the true nature of this disease, the physiological function of zinc, and kindred questions. One approach to the solution of these problems lies in determining the nutrient and environmental conditions under which this disturbance can be developed artificially. Except for some indications recently reported by Haas (*3*) previous attempts to produce mottle-leaf of citrus under the known conditions of solution or sand culture have been unsuccessful. Hoagland, Chandler, and Hibbard (*4*) have produced little leaf of apricot trees and zinc-deficiency symtoms in other plants by the omission of the zinc from culture solution, when special efforts were made to minimize zinc contamination. However, trials with citrus gave negative results under similar conditions.[3] This, they thought, might in part be due to the low light intensities prevailing at Berkeley, Calif.

Accordingly, a series of experiments with citrus were set up at Riverside, Calif., in March 1936. The positive results of these trials, together with several interesting observations, appear to justify a progress report at this time.

DESCRIPTION OF EXPERIMENTS

The technique of these experiments is given in some detail for the benefit of those engaged in similar or related studies.

Leafy twig cuttings from healthy, vigorous, Valencia orange trees were rooted in coarse-grained sand and then transferred to pyrex-glass battery jars containing nutrient solutions of the desired composition. The cuttings were held in place by specially painted wooden plugs inserted in appropriately spaced holes in Bakelite covers. The sugar pine plugs were first painted with a high-grade asphaltum varnish and then with two coats of a synthetic Bakelite-type varnish. Previous work has shown that both Bakelite and the varnishes used are highly resistant to the deteriorating action of weak salt solutions and at the same time yield no toxic products to the culture solution. Although the ash of these materials contained traces of zinc and various other inorganic constituents (table 1), the resinous matrix of

[1] Received for publication Feb. 23, 1937; issued September 1937. Paper no. 366, University of California Citrus Experiment Station and Graduate School of Tropical Agriculture, Riverside, Calif.

[2] Reference is made by number (italic) to Literature Cited, p. 378.

[3] Unpublished observations communicated to the authors by D. R. Hoagland.

TABLE 1.—*Impurities in major chemicals (c. p. grade) and accessory materials*

Material	Manufacturer or agent	Lot no.	Impurities as determined spectrographically		
			Zinc content	Definite traces	Just detectable
			P. p. m.		
K_2SO_4	General Chemical Co.	5	3.5		Bi, Ca, Cu, Mg, Pb, Sn, Si.
KNO_3	J. T. Baker	41834	1.0	Bi	Ca, Cu, Pb, Sn, Si.
$Ca(NO_3)_2 4H_2O$	Merck	30553	1.0	Ag, Ca, Fe, Pb, Sn	Bi, B, Cu, Mg, Si.
$CaSO_4.2H_2O$	do	31794	1.0	Cu, Pb	Ba, Bi, Mg, Ni, Si, Sn.
K_2PO_4	J. T. Baker	11623	3.5	As, Bi, Ca, Cu, Mg, Pb, Sb, Si, Sn.	Ag, Hg.
$MgSO_4.7H_2O$	Merck	31433	2.0	Ag, Cu, Mg	Bi, Pb, Sn.
$Mg(NO_3)_2.6H_2O$	J. T. Baker	41934	8.0	Cu, Mg, Pb, Sn	Bi, Si.
$FeSO_4$	do	72426	20.0	Cu, Bi, Mg, Pb, Si, Sn	Ti.
KOH	do	121521		Ca, Na	Sr.
$KHSO_4$	do	51530		Ca.	
NaOH	Mallinckrodt	1177126		Al, Ca	Fe, K, Mg, Sr.
Bakelite	Bakelite Corporation.			Al, Ca, Cu, Mg, Zn.	
Asphalt paint (Gila coat).	Fuller & Co			Ca, Pb, Ag, Zn.	
Phenolic resin varnish.	Du Pont			Pb, Ca, Co, Cu, Mg, Mn.	Al, Fe, Ni, Si.

the paint prevents appreciable solution of these elements. Before being used the battery jars, Bakelite covers, and painted plugs were digested with 20-percent hydrochloric acid. In order to avoid contamination of the nutrient solution with dust from the air, the covers were held tightly to the ground edge of the battery jars by springs attached to a metal harness surrounding the jars. The battery jars were coated on the outside with an asphaltum, followed by an aluminum varnish. During the summer it became necessary to reduce the solution temperatures within the jars by insulation with sphagnum moss. A portion of the general set-up is shown in figure 1.

All water used in the experiment was redistilled in an all-pyrex-glass still designed for continuous operation.

Zinc was quantitatively determined in all of the major chemicals used by the method of Vanselow and Laurance (*11*). With the exception of the iron sulphate, they were found to contain so little zinc that purification was not attempted (table 1).

Because of the precautions taken to prevent contamination and the fact that plant stimulation has often been produced by many other minor elements, 54 trace elements were added as a basic treatment to most of the cultures. A moderately concentrated stock solution of each was made up separately, and from these, secondary mixtures of compatible combinations.

The kinds and amounts of these minor elements added to the final culture solution, the source from which derived, and the results of a qualitative spectrographic determination [4] of the impurities contained by these chemicals are given in table 2.

The impurities carried by the major salts and several accessory compounds are shown in table 1. All acids used in the experiments were redistilled in a pyrex-glass still.

[4] These determinations were made by means of a concave grating spectrograph (*11*), samples of the dry material being arced on graphite rods.

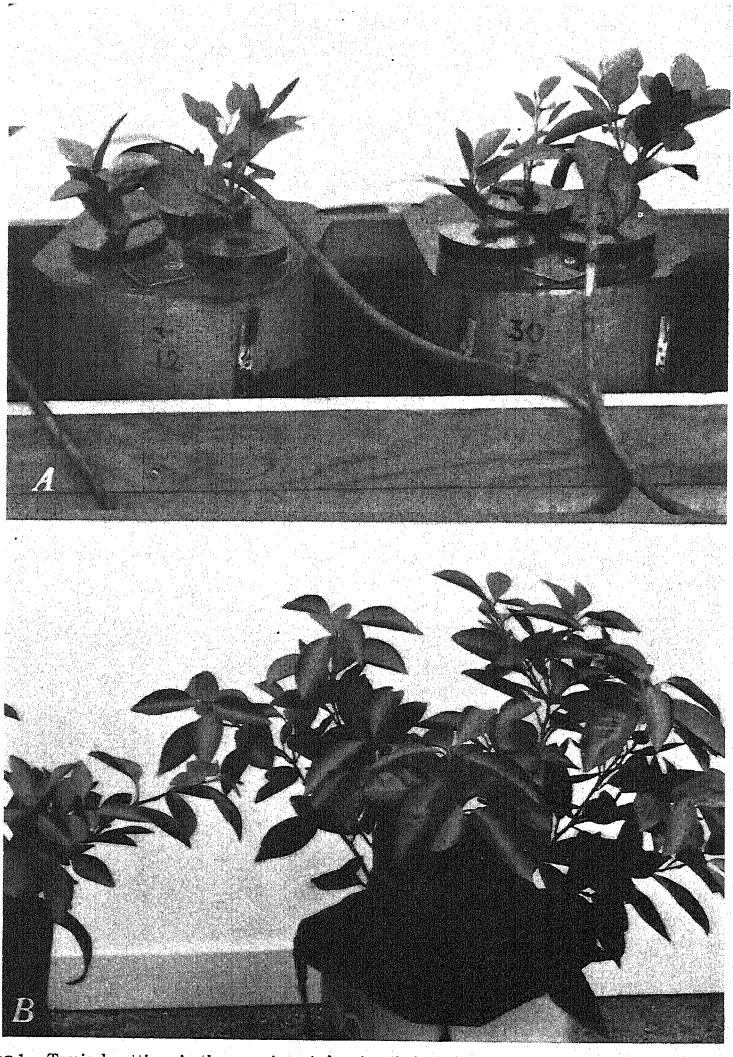


FIGURE 1.—Typical cuttings in the experiment showing their size after emergence of first growth cycle (*A*) and again after third growth cycle (*B*).

TABLE 2.—*Trace elements added to culture solution, and impurities in chemicals from which derived*

Element	Amount added to final culture solution		Source	Manufacturer or agent	Grade	Lot no.	Impurities as shown spectrographically	
	Per liter	γ At.[1]					Definite traces	Just detectable
	Milligrams							
Al	0.539	20.0	$KAl(SO_4)_2.12H_2O$	J. T. Baker	C. p	10722		Ca.
Sb	.012	.1	Sb_2O_3	do	do	11629		Al, Ca, Mg, Pb, Si.
As	.007	.1	As_2O_3	do	do	8226	Sb	Ca, Si.
Ba	.686	5.0	$BaCl_2.2H_2O$	do	do	12231	Ca, Sr	Cu, Fe, Mg, Ni, Pb, Si.
Be	.090	10.0	$Be(NO_3)_2.3H_2O$	Eimer & Amend	do	H100	Ca, Mg, Si	Ba, Na, Ti.
Bi	.021	.1	Metal	Coleman & Bell	do			Ca, Pb, Si.
B	.541	50.0	H_3BO_3	Merck	do	30343		Si.
Br	1.598	20.0	NaBr	J. T. Baker	do	61522	Ca	Al, Ba, Sr, Ti, V.
Cd	.011	.1	Metal					Ca, Cu, Mg, Pb, Si.
Ce	.014	.1	$CeCl_3$	Kahlbaum		X2C1	Ca, Mg, Na, Sr	Cu, Si, Sn, Th, V.
Cs	.013	.1	CsCl	do		Z1309	Na, Rb	Ca, K, Mg, Sr.
Cl	7.092	200.0	NaCl	J. T. Baker	C. p	8530	Mg	Ba, Ca.
Cr	.052	1.0	$K_2Cr_2O_7$	Merck	do	30793	Ca	Co, Pb, Sr.
Co	.005	.1	$CoCl_2.6H_2O$	J. T. Baker	do	31227	Ca, Mn, Ti, Ni	Cu, Fe, K, Mg, V.
Cb	.009	.1	Cb_2O_5	A. P. Vanselow			Pt, Si, Ti	Ca, Cr, Cu, Mg, Sn, Ta, W, Zr.
Cu	.063	1.0	$CuSO_4.5H_2O$	Merck	C. p	30663	Ni	B, Ca, Fe, Mg, Pb, Si.
Er	.050	.1	$Er(NO_3)_3xH_2O$	Eimer & Amend	do	A27	Al, Ho, Lu, Mg, Pb, Zn, Ca, Dy, Tm , Yt, Yb	Ba, Ce, Cu, Nd, Sm, Si, Sn.
F	.019	1.0	NaF	J. T. Baker	do	81622	Ca, Sr	Al, Ba, Cu, Mg, Pb, Sn, Ti.
Ga	.006	.1	Metal	A. D. Mackay			Cu, Mg, Ti, Tl, V, Zn	Al, Ba, Ca, Si, Sn, Sr.
Ge	.007	.1	GeO_2	Eimer & Amend		B36	Al, Ba, Ca, Mg, Na, Si	Cu, Fe, Sr, Ti.
Au	.019	.1	$AuCl_3$	University of California			Ca, Si	Cu, Mg.
In	.011	.1	Metal	A. D. Mackay			Cu, Pb, Zn	Ag, Al, Ba, Bi, Cu, Ga, Mg, Si, Sr.
I	.012	.1	NaI	Merck	Reagent	30254		Ca, Mg.
Ir	.019	.1	$IrCl_3$	Eimer & Amend		D37	Fe, Ru, Sr, Ti, Ca	Ag, Al, Ba, Co, Cu, Mg, Ni, Pt, Si.
La	.013	.1	$LaCl_3$	do	C. p	A27	Ca, Fe	Cu, Pb.
Pb	.020	.1	$2PbCO_3.Pb(OH)_2$	J. T. Baker	do	1019	Ca	Al, Mg, Si.
Li	.069	10.0	$LiCO_3$	do	do	111322	Ca, Sr	Al, Fe, Na, Mg, Ti.
Mn	.549	10.0	$MnCl_2.4H_2O$	Eimer & Amend	Tested purity	H94	Ca	Al, Ba, Cu, Fe, Mg, Ni, Pb, Si, Sn, Sr.
Hg	.020	.1	$HgCl_2$	Merck	C. p	30692		Ca.
Mo	.009	.1	MoO_3	J. T. Baker	do	11429	Ca	W.
Nd	.014	.1	$NdCl_3$	Eimer & Amend	do	C47	Ca, Dy, Pr, Sa, Tm	Ag, Al, Ce, Cu, Er, Gd, La, Mg, Sc, Si, Ta, Zr.
Ni	.005	.1	$NiCl_2.6H_2O$	do	Tested purity	B38	Ca, Mn, Dy, Ti, Co	Cu, Fe, Mg, Si.
Os	.019	.1	OsO_4	do				

Pd	.610	.1	$PdCl_2.2H_2O$	A. H. Thomas Co.			Ca, Cu, Au	Ag, Fe, Rh, Mg, Si, Ru.
Pt	.019	.1	K_2PtCl_6	Eimer & Amend	C. p.	E21	Ca	Ag, Al, Au, Si, Ti, V.
Rh	.010	.1	$RhCl_3$	?	?	?	Ag, Ca	Cu, Si, Ti, Zr, Mg.
Ru	.010	.1	$RuCl_3$	?	?	?	Ca, Ti, Zn, Rn?	Ag, Al, Cu, Fe, Rh, Mg, Si, V, Ta.
Rb	.008	.1	Rb_2SO_4	Eimer & Amend	C. p.	D45	K	Ca, Mg, Na.
Se	.079	1.0	Na_2SeO_3	E. H. Sargent	do		Ca	Al, Mg, Pb.
Si	1.400	50.0	SiO_2	J. T. Baker	do	72527	Al, Fe, Mg, Na, Ti	Ba, Ca, Cu, Sr.
Ag	.010	.1	Ag_2SO_4	do	do	32927		Ca, Mg, Si.
Na	4.600	200.0	NaCl	do	do	8530	Mg	Ba, Ca.
Sr	.087	1.0	$SrCO_3$	Cenco	do	1223	Ca, Ba, Mg	Cu, Mn, Na, Si.
Ta	.018	.1	Metal	A. D. Mackay			Ca, Si	Al, B, Ni, Ti.
Te	.012	.1	Na_2TeO_3	Eimer & Amend	Pure	C97	Ag, Al, Ca, Cu, Pb	Ba, Fe, K, Mg.
Tl	.020	.1	$TlNO_3$	Cenco	C. p.		Fe, Mg	Al, Ca, Cu, Mn, Pb.
Th	.023	.1	$ThCl_4$	Will Corporation	do		Zr	Ca, Ce, Cu, Mg, Si, Ti.
Sn	.011	.1	$SnCl_2.2H_2O$	J. T. Baker	do	22230	Pb	Al, Ca, Cu, Fe, In, Mg, Si.
Ti	.239	5.0	TiO_2	Eimer & Amend	do	B38		Ca, Cu, Mg, Pb, Si.
W	.018	.1	WO_3	J. T. Baker		4126		Ba, Ca, Cr, Cu.
U	.023	.1	$UO_2.(C_2H_3O_2)_2.2H_2O$	do	C. p.	102333	Mg, Th	Ba, Ca, Na, Sn, Zn.
V	.059	1.0	NH_4VO_3	Eimer & Amend	do	B36		B, Ba, Ca, Si.
Yt	.008	.1	$Yt(NO_3)_3.6H_2O$	do		D37	Ba, Dy, Er, Sm, Zr, Ca, Yb.	Al, Ce, Gd, Pb, Cu, Fe, Mg, Nd, Si, V.
Zr	.009	.1	$Zr(NO_3)_4.5H_2O$	do	C. p.	D123	Ca, Si, Ti	Mg, Pb.

[1] One millionth of a gram-atom.

The field observation that both nutrient and light conditions apparently modify the zinc requirement of citrus suggested that the cultures with and without zinc be duplicated under such conditions as to render both of the above factors variable. The treatments as finally set up are given in table 3. The cultures designated as "low light"

TABLE 3.—*Nature of treatments under different light intensities*

Light intensity	Nature of culture solution		Culture no.
	Major elements	Minor elements	
Low (about 60 percent less than out of doors[1]).	Low NO_3	All,[2] no zinc	1 and 2.
		All, plus zinc (0.65 p. p. m.)	5 and 6.
	High NO_3	All, no zinc	9 and 10.
		All, plus zinc (0.65 p. p. m.)	13 and 14.
High (about 20 percent less than out of doors).	Low NO_3	All, no zinc	3 and 4.
		All, plus zinc (0.65 p. p. m.)	7 and 8.
	High NO_3	All, no zinc	11 and 12.
		do	21 and 22.
		All, plus zinc (0.65 p. p. m.)	15 and 16.
		None except Mn, B, Na, Cl, no zinc.	17 and 18.
		Double strength, all, no zinc	19 and 20.
	High NO_3 (purified by Steinberg method).	All, no zinc	23 and 24.

[1] Measured with Weston photographic exposure meter.
[2] Designates 54 trace element combination given in table 2.

were placed under a small lath shelter within the greenhouse, where the light intensity was reduced to about one-half that prevailing in other parts of the greenhouse. To secure information on the combined effects of the trace elements, cultures with no minor elements present except iron, manganese, boron, sodium, and chlorine, and with double the amounts of all but iron were included. Also, two cultures were purified according to the Steinberg (*10*) method by boiling with an excess of calcium carbonate. (Only the major salts were present at the time of purification, the trace elements being added to the purified solution.)

The composition of the culture solution as regards the major elements is shown in table 4. Iron sulphate, from which the zinc had

TABLE 4.—*Major components of low- and high-nitrate culture solutions*

Type of solution	Salt used	Quantity per liter	Quantity of indicated elements or radicals per liter					
			Ca	Mg	K	NO_3	SO_4	PO_4
		Grams	*Milligram-atoms*	*Milligram-atoms*	*Milligram-atoms*	*Milligram-atoms*	*Milligram-atoms*	*Milligram-atoms*
Low nitrate	K_3PO_4	0.0212			0.30			0.10
	$Ca(NO_3)_2.4H_2O$	.2395	1.00			2.0		
	$CaSO_4.2H_2O$	.1331					0.77	
	$MgSO_4.7H_2O$	.1750	77	0.71			.71	
	K_2SO_4	.1768			2.03		1.01	
	Total		1.77	.71	2.33	2.0	2.49	.10
High nitrate	K_3PO_4	.0212			.30			.10
	$Ca(NO_3)_2.4H_2O$	1.4848	6.27			12.55		
	KNO_3	.4974			4.92	4.92		
	$Mg(NO_3)_2.6H_2O$	.3244		1.26		2.53		
	$MgSO_4.7H_2O$	.6162		2.50			2.50	
	Total		6.27	3.76	5.22	20.00	2.50	.10

been removed by precipitation with hydrogen sulphide, according to the Vanselow and Laurance (*11*) method, was added daily at the rate of 0.2 mg iron per liter.

Previous experiments by Haas (*2*) with Valencia orange cuttings have indicated that an intermittent rather than a continuous supply of phosphate is conducive to better growth.

Accordingly, throughout the early stages of this experiment, phosphate was alternately added and withheld biweekly. The cultures were aerated continuously, the compressed air being introduced through sintered glass disks fused into the ends of pyrex-glass tubes.

By the addition either of nitric acid or potassium hydroxide, the pH of the solutions was kept between 6.5 and 7.0.

The culture solutions were at first renewed every month, and later every 3 weeks. Water lost by transpiration and evaporation was replaced daily by the addition of redistilled water. After the experiment had progressed for some time, quantitative determinations of zinc were made in both the fresh and the used solutions. The results of these determinations are as follows:

	Zinc per liter, miligrams
Fresh solution	0. 007
Used solution (1 month old)	. 003

RESULTS

The cuttings were transplanted from the propagating bed into the culture solutions on March 18, 1936, after the roots had been thoroughly rinsed in distilled water. In less than 12 days new roots began to develop and shortly thereafter new shoots appeared. By April 28 the leaves and shoots of the first growth cycle had attained full size, though the leaves were as yet somewhat tender and immature. None of the new leaves showed signs of mottle-leaf at this stage. The low-light plants appeared somewhat more healthy than the high-light plants and those grown in high nitrate were more vigorous than those grown in low nitrate.

By June 17 a second cycle of growth had emerged, the leaves and shoots having reached about the same stage of maturity as that of the first cycle on April 28. In contrast to the appearance of first growth cycle at this stage, the leaves of the plants in the cultures without zinc were more yellowish, some of them suggesting nitrogen deficiency; others showed some venation as in iron deficiency. Characteristic, though faint, mottle-leaf markings had developed in many of the now mature first-cycle leaves of the plants growing in minus-zinc, high-light cultures. There was no mottle, however, in any of the plants of the low-light series nor were any of the plants of cultures receiving zinc affected. By the middle of July the second growth cycle was virtually mature, and the leaves, which on June 17 had appeared yellowish, had become strikingly mottled. The plants of cultures 9 and 10 (low-light, high-nitrate, no-zinc) were only slightly mottled as compared with the more severe condition of corresponding cultures subjected to the full light of the greenhouse.

After the first 2 months of this experiment the roots of some of the plants in various cultures began to rot. This type of rot (gelatinization) always began on the very youngest roots, gradually spreading to older roots. At no time during this experiment was it possible to

correlate positively the incidence of this root rot with zinc, nitrate, light, or trace-element variables; on the other hand, it became increasingly evident as the experiment progressed that there was some relation between the phosphate status of the nutrient medium and the root rot. When phosphate was present the rotting usually became intensified, whereas when phosphate was omitted it tended to remain unchanged. In an effort to control the root rotting it was decided in June to decrease the concentration of phosphate to one-half of that employed at the outset.

Toward the latter end of July a third growth cycle began to emerge on all those plants whose roots were still in a healthy condition. In contrast to the early chlorotic appearance of the second growth cycle, this new cycle was green and vigorous. Moreover, the mottled leaves of affected plants began to turn green. A possible phosphate-zinc relation being suspected, phosphate was restored to its original level and kept present continuously until the termination of the experiment on September 15. As a result of this latter change, the leaves of the new third cycle soon began to acquire a yellowish cast, the venation of some of them becoming marked. By the middle of September the third cycle growth was virtually mature, the leaves of the plants in the minus-zinc cultures having become typically mottled.

Inasmuch as the roots of all save those in one of the plus-zinc, high-light cultures began to rot at the time the second change in phosphate technique was inaugurated, it was decided to make frequent small additions of zinc to one culture (no. 24). In contrast to the behavior of all the plants grown in the solutions lacking zinc, the third growth cycle of the plants in this culture remained healthy, the leaves attaining full size and becoming fully green. Moreover, the leaves of the second cycle, which on July 15 were badly mottled, became fully green. Representative leaves from the first, second, and third growth cycles of a plant from culture no. 24 are shown in figure 2. The first growth-cycle leaves (*C*) were at no time more than faintly mottled. The second-cycle leaves, *B*, formerly severely mottled, became completely green though there was no increase in leaf size.[5] The leaves *A* from the third cycle of growth were fully green and normal sized.

Owing to the increasing incidence of root rot in cultures hitherto healthy (occasioned, no doubt, by the continued presence of phosphate) the experiment was terminated on September 15.

An idea as to the growth made by healthy cuttings during the 6 months of this experiment may be had by reference to figure 1, *B*. Pictures of a few leaves from minus-zinc cultures showing some of the variations on leaf markings and sizes are presented in figure 3. The top row (*A*) shows mildly affected leaves taken from plants grown under low light conditions. The leaves in figure 3, *B*, show the more pronounced mottling developed under high light. That these leaves represent typical mottle-leaf may be seen by comparison with those shown in figures 4 and 5.[6]

The production of mottle-leaf under controlled conditions has made it possible to observe in detail the various stages in the development

[5] The greening of mottled leaves following zinc addition to the culture medium accords with the observation of Parker (*8*) and Reed and Parker (*9*) on the effects of zinc sulphate sprays.

[6] At the authors' request these latter were picked from orchard trees by E. R. Parker. At the time they were selected, the corresponding foliage of adjacent zinc-sulphate-treated trees was virtually free from leaves with markings such as those depicted in figs. 4 and 5.

of what is commonly regarded as the typical mottle-leaf pattern. The immature stage of leaves destined to become mottled is often characterized by a pronounced venation, the general color of the leaf being yellowish (fig. 6, two top leaves). As the leaf matures, the green areas adjacent to the veins spread and deepen in color and the

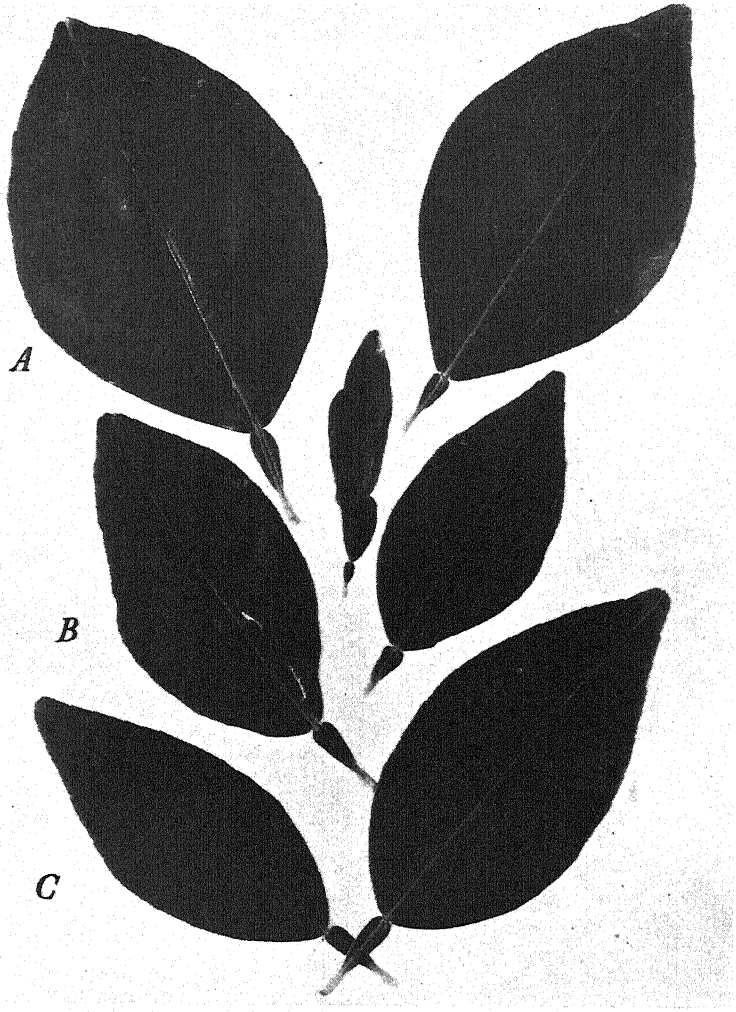


FIGURE 2.—Leaves from plant of culture no. 24: *A*, Third-cycle leaves which emerged green and healthy following zinc additions; *B*, second-cycle leaves formerly severely mottled, but caused to turn green following the addition of zinc; *C*, first-cycle leaves.

yellow-green areas between the veins become more pronouncedly yellow, thus giving rise to strikingly "mottled" patterns, often quite variable but with an element of similarity throughout. Small green spots are often seen in the yellowed areas. In more severe stages, the immature leaves are often uniformly yellow, the lower portion

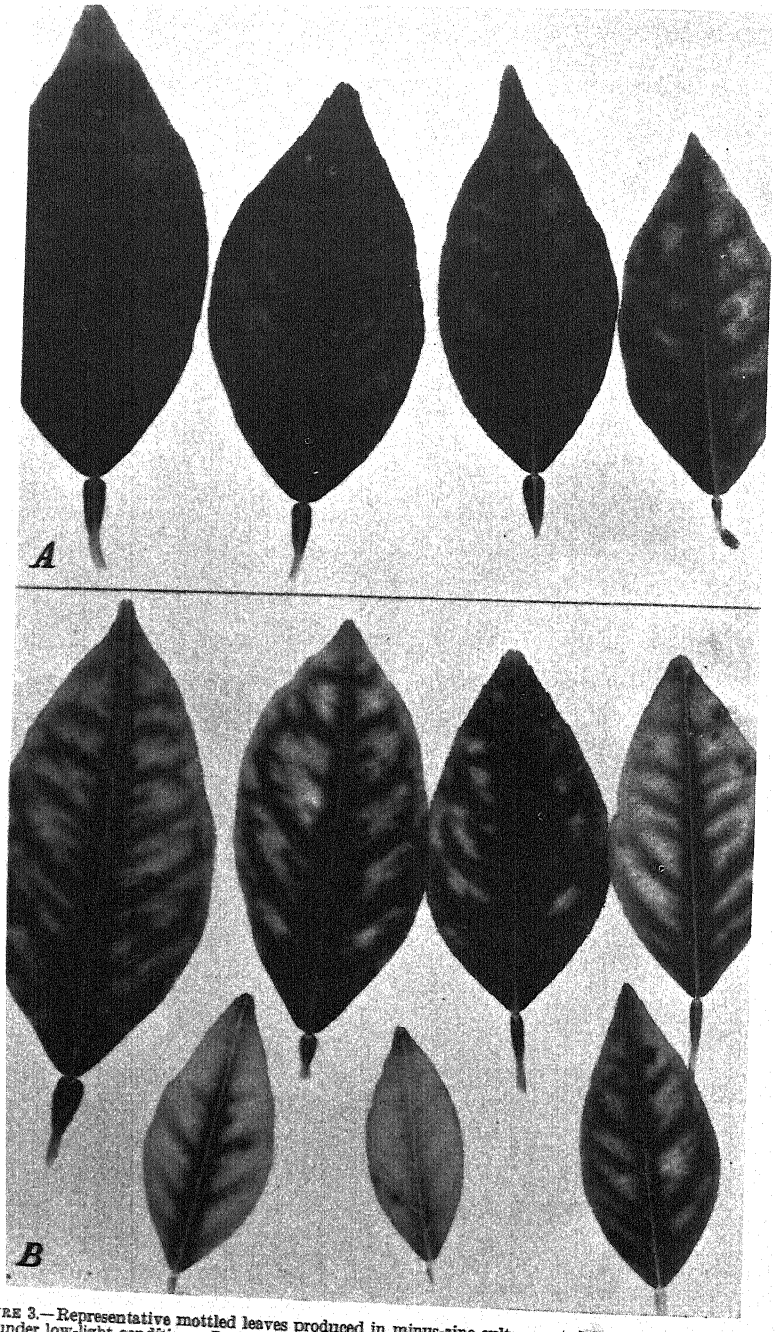


FIGURE 3.—Representative mottled leaves produced in minus-zinc cultures: *A*, Mild mottling produced under low-light conditions; *B*, the more severe mottling produced under higher light intensities.

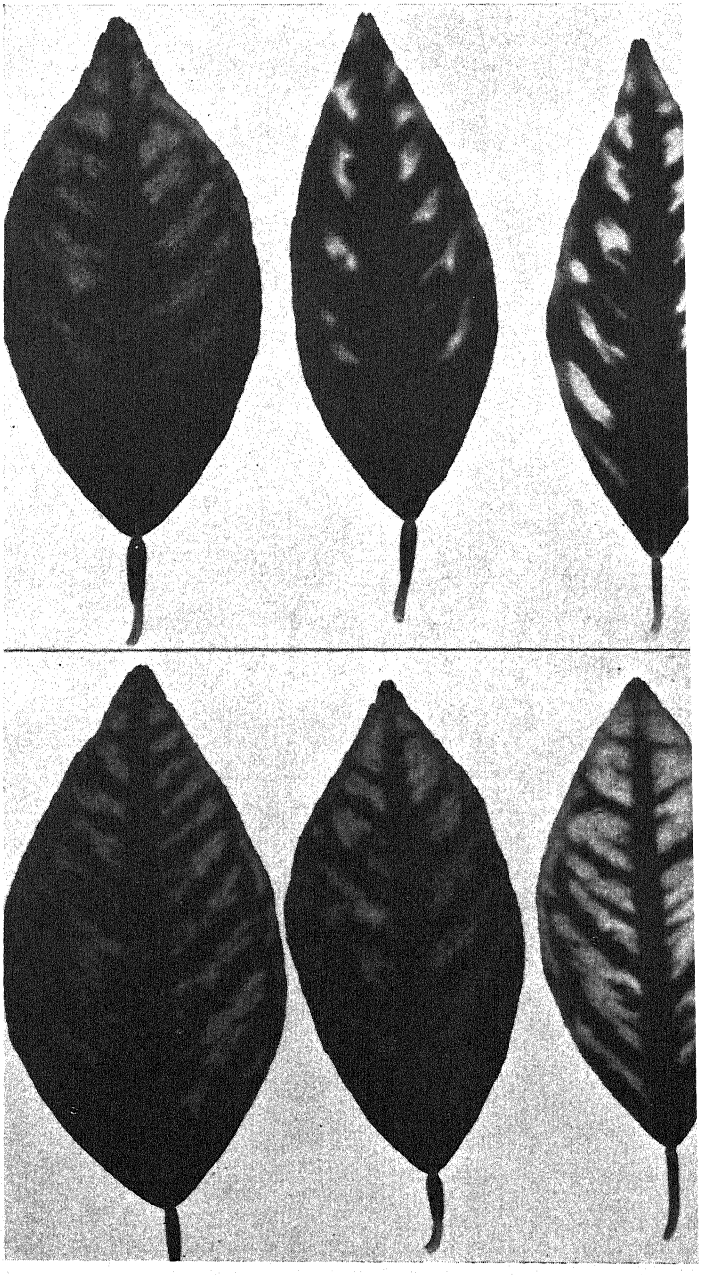


FIGURE 4.—Leaves from mottle-leaf-affected trees, showing some of the variations in size and

of the veinal system being the only green part of the mature leaf. In mild cases the new growth may emerge entirely green, and only as the leaf matures will small yellow-green blotches appear.

During the course of leaf development, variations in degree of mottling may be induced by nutritional changes. Thus the continuous presence of phosphate under the conditions of this experiment

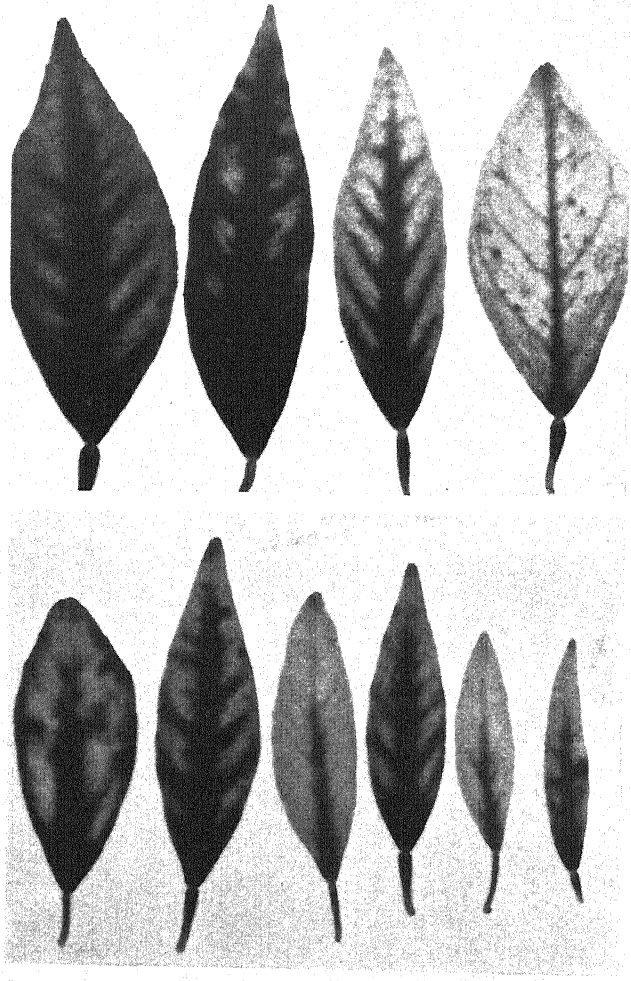


FIGURE 5.—Leaves from mottle-leaf-affected trees showing some of the variations in size and markings

caused immature but full-sized green leaves to mottle as they matured. On the other hand the frequent addition of small amounts of zinc caused mottled leaves to become green. However, at no time during the course of these experiments did any of the fully mature green leaves of the original cuttings become mottled, despite the development of

pronounced mottle-leaf in subsequent new growth. Cases of a similar nature on mature trees growing in orchards are often seen. This suggests that the zinc of mature leaves is rather immobile, as in the case of iron.



FIGURE 6.—Progressive stages in the development of typical mottle-leaf. Two top leaves show immature stage, the others progressive stages as the leaf matures.

It is of interest to note the effects of the trace elements. Where used in double strength, as indicated in table 3 (cultures nos. 19 and

20), top and root growth was definitely depressed though the leaves showed no localized evidence of injury. The roots were somewhat brownish and stubby. The plants of those cultures receiving zinc in addition to the 54 trace elements were healthy in appearance and showed no evidence of injury. It is unfortunate that, for purposes of comparison, cultures with zinc but minus the trace elements were not included in this experiment.

Despite the high boron content of pyrex glass plus the addition of 0.5 p. p. m. of this element to the culture solution, definite, though not severe, symptoms of boron deficiency (*1*) appeared in some of the plants during the course of the experiment.

CONCLUSIONS

Under the conditions of this experiment it has been possible to produce mottle-leaf of citrus by omitting zinc from the culture solution. Conversely, the addition of zinc not only prevented the development of mottle-leaf, but small amounts of zinc supplied frequently to plants already affected with mottle-leaf brought about recovery. Plants grown in the full light of the greenhouse (intensity about 80 percent of that out-of-doors) became severely mottled, whereas those subjected to a lower light intensity (about 40 percent of that out-of-doors) were but slightly affected. These results are in accord with the field observation that the leaves on the south side of citrus trees are usually more mottled than those on the north side.

During the early stages of this experiment there were definite evidences that the plants grown under conditions of high nitrate were more mottled than those grown with low nitrate, though in the latter there was no evidence of nitrogen starvation. However, the gradual decrease in the numbers of healthy cultures, owing to the incidence of root rot, has left this question unsettled.

Apparently the degree of mottling can be strongly influenced by the phosphate content of the nutrient medium. Increases in phosphate accentuate the mottling. Whether this effect is due to decreased zinc solubility in the culture solution, or to decreased availability within the plant, cannot be stated definitely. Olsen (*6*) has shown that high phosphate will induce iron chlorosis in certain plants through reactions within the plants. Zinc may be similarly affected.

Although mottle-leaf and root rotting were both accentuated by the continued presence of phosphate, there seems to be no necessary relation between these two conditions. On the one hand, mottle-leaf developed in plants that at no time were affected with root rot; on the other, root rot occurred unaccompanied by mottle-leaf.

The results of this experiment are more in harmony with the view that zinc is an indispensable plant food element, mottle-leaf of citrus being a manifestation of this deficiency, than that this element functions as an antiseptic or corrective.

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NUTRITIVE VALUE FOR DAIRY COWS OF ALFALFA HAY INJURED BY SULPHUR DIOXIDE[1]

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INTRODUCTION

In the vicinity of some industrial plants, the concentration of sulphur dioxide in the atmosphere becomes sufficiently high to injure the vegetation. Alfalfa (*Medicago sativa* L.) is one of the plants that is most susceptible to injury from this gas.

The injury to plants is of two types, chlorotic and acute (*5*).[3] The chlorotic type, which appears several days after fumigation, is evidenced by a rather rapid disappearance of chlorophyll from an otherwise apparently normal leaf. The acute type sometimes results in markings of the alfalfa leaves within the hour, the leaves first stiffening and then becoming flaccid. The flaccid areas of the leaves bleach almost to an ivory color after exposure to a few hours of sunshine. The injury is first noted at the margin of the leaflet, and progresses between the veins toward the center. Leaves frequently drop from the plant when the greater part of the green tissue of the leaflets is destroyed. The injury with which this paper deals was mostly of the acute type, but there had been little, if any, dropping of the leaves at the time the alfalfa was cut.

The question has been asked by farmers and others living in areas where the alfalfa crop is sometimes affected by sulphur dioxide whether the injury to the leaflets reduces the feeding value of the alfalfa. If the feeding value is reduced it is important to know whether the reduction is due to lessened palatability, to decreased digestibility, or to an actual diminution in the nutrient value of the plant. In the fall of 1930, alfalfa fumigated by sulphur dioxide became available and the opportunity was presented to study the problem by conducting a 90-day double-reversal feeding trial.

EXPERIMENTAL MATERIALS AND METHODS

A field of fourth-cutting alfalfa which had been noticeably marked by sulphur dioxide on three occasions and subjected to relatively high concentrations on numerous other occasions, was selected for the experiment. The alfalfa was grown between the approximate dates of August 3 and September 8, 1930, on a 20-acre field in the upper Rio Grande Valley, situated about 4 miles northwest of the plant of the El Paso Smelting Works at El Paso, Tex.

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[2] The authors acknowledge the assistance of the following persons during the course of the experiment: L. V. Olson, for the photograph shown as fig. 3 and data for the curves in fig. 2; Dr. M. D. Thomas, for sulphur analyses of the two hays; G. R. Quesenberry, for grading the hays; Dr. J. N. Abersold, for energy determinations on the feeds, refuse, and excreta; W. H. Ball, for the data used in making fig. 1; and Fred Wolf, John Gaume, and Maggie McKeand, for assistance in making the many calculations involved.

[3] Reference is made by number (italic) to Literature Cited, p. 391.

Before the alfalfa to be used for the experiment was cut, the percentage of the leaflets that were marked by the gas was determined. The calculations were made from the averages of actual counts of all the marked and all the normal leaflets within the area of a quadrat of 4 square feet at definite intervals diagonally through the field, and from estimates based on these counts. The injury varied greatly in different parts of the field, as is shown in figure 1. Variation in the

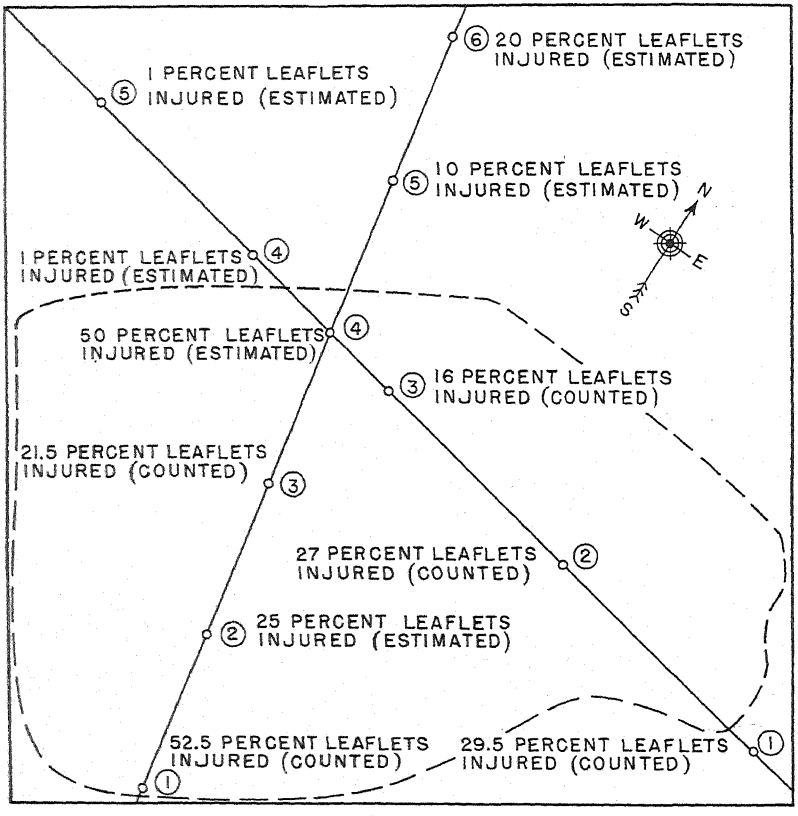


FIGURE 1.—Alfalfa field, showing location of points at which injury was estimated and area (within dotted lines) from which the experimental hay was obtained.

intensity of injury is not unusual even in limited areas, and is sometimes very marked over larger areas. This is thought to be due to air currents which vary the concentration of the gas, to the density of vegetation which absorbs the gas, and to differences in soil moisture. The alfalfa used for the experiment was taken from the south portion of the field only (the area within the dotted lines in fig. 1), where more than 25 percent of the leaflets were acutely marked and the injury was most conspicuous.

The smelter was in continuous operation while the experimental alfalfa was growing, and during a large part of the time the wind was from the southeast and carried sulphur dioxide to the field. Records from an automatic sulphur dioxide recorder (7) located about 0.7

mile nearer the smelter stack, are believed to show the approximate durations of exposure and concentrations of sulphur dioxide to which this field was subjected (fig. 2).

Owing to a burned-out fuse, the records for August 21 and 22 are incomplete, but the field is known to have been noticeably marked

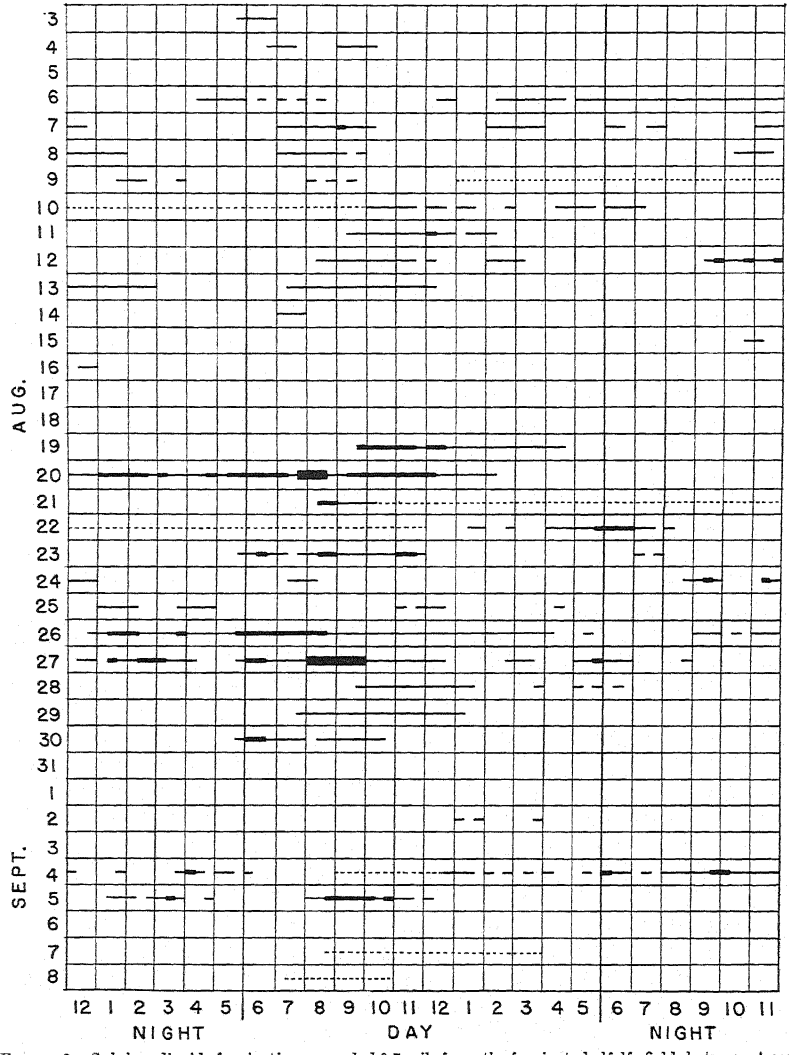


FIGURE 2.—Sulphur dioxide fumigations recorded 0.7 mile from the fumigated alfalfa field, between August 3 and September 8, 1930. Blank spaces indicate no sulphur dioxide; light lines, traces; medium lines, light concentrations; heavy lines, concentrations strong enough to mark vegetation; and dotted lines indicate recorder not working.

on those days. On August 27 the heaviest fumigation occurred. This was 12 days before the hay was cut.

Figure 3, an aerial photograph, shows the relation of the recorder and the smelter stack to the field from which the fumigated alfalfa

was obtained. It may be noted that the recorder is nearly on an air line between the south corner of the field and the smelter stack. The photograph shows also the type of topography surrounding this narrow cultivated valley, which in part accounts for the varied air currents.

For control purposes, fourth-cutting alfalfa grown during the same period in a field more than 30 miles north of the smelter was used. This hay was selected by a Government hay grader to match the fumigated hay as nearly as possible, except for sulphur dioxide markings. The field from which the hay was obtained is located more than 20 miles beyond the most remote point from the smelter where even traces of sulphur dioxide markings have been observed, so that it is reasonable to assume that the plants had not been subjected to sulphur dioxide gas.

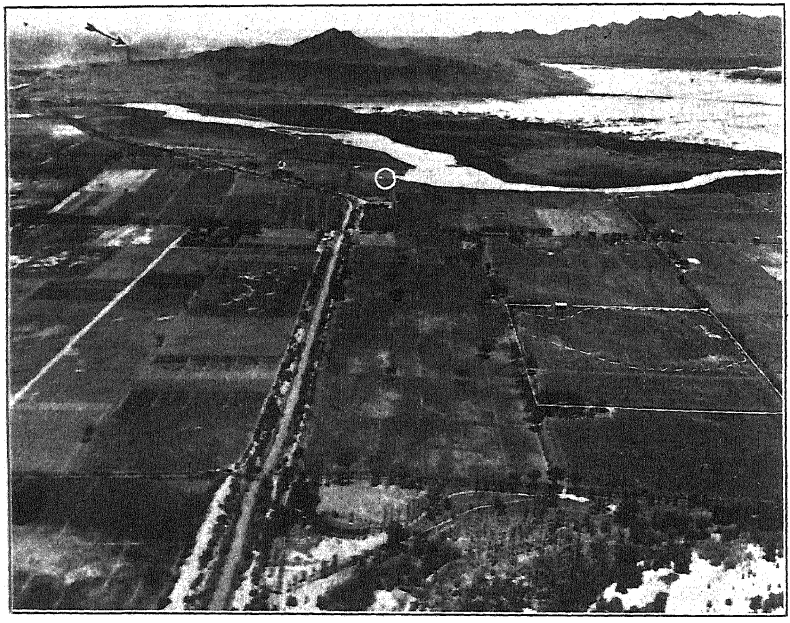


FIGURE 3.—Aerial photograph showing relative positions of the fumigated field (within square), gas recorder (in circle), and smelter smokestack (indicated by arrow) in a narrow irrigated valley. The alfalfa fumigated with sulphur dioxide was obtained from within the area enclosed by the dotted line.

Both lots of alfalfa as finally selected were graded U. S. No. 2 Extra Leafy. The fumigated hay was found to consist of 42.03 percent of stems and 57.97 percent of leaves by weight. The check hay contained 46.93 percent of stems and 53.07 percent of leaves. The grader stated that the market value of the check hay was about 5 percent higher than that of the fumigated hay, principally on account of the difference in color.

The double-reversal feeding and digestion trial of 90 days' duration was conducted with 10 lactating cows. All of the cows were excellent producers. It will be noted from table 1 that five of them were not pregnant at the end of the experiment, and only one had carried a fetus for more than 68 days. Eckles (*2, pp. 413–415*) has shown that

the first 150 days of pregnancy do not materially affect the rate of milk production.

TABLE 1.—*Description of cows used in the fumigated alfalfa experiment*

Cow no.	Breed	Age at close of experiment	Duration of pregnancy at close of experiment	Time from freshening date to close of experiment	Cow no.	Breed	Age at close of experiment	Duration of pregnancy at close of experiment	Time from freshening date to close of experiment
		Days	*Days*	*Days*			*Days*	*Days*	*Days*
16	Holstein-Friesian	1,861	68	156	22	Jersey	1,647	[1]	368
31	do	1,183	162	206	45	Guernsey	867	[1]	129
42	do	958	40	232	24	do	1,500	[1]	167
41	Guernsey	975	[1]	139	32	Holstein-Friesian	1,168	50	198
18	Jersey	1,791	[1]	393	40	do	991	8	250

[1] Not pregnant.

The cows were divided into two groups of five each. One group was fed the fumigated hay and the other was fed the check hay, the hays being reversed at the end of each 30-day period. Otherwise the rations of the two groups were identical and consisted of a good grade of silage made from Mexican June corn, a grain mixture consisting of 3 parts of ground no. 2 mixed corn, 1 part of wheat bran, 1 part of cottonseed, and 1 part of cottonseed meal (43-percent protein). It is the practice in this vicinity to feed silage and a grain ration with the alfalfa, and this was done by dairymen who were concerned with alfalfa marked by sulphur dioxide.

The time was divided into three periods of 30 days each. Digestion trials were conducted during the last 10 days of each 30-day period. Three of these 10 days were allowed for the cows to become accustomed to the conditions of the digestion trials; the remaining 7 days were used as a collection period.

The alfalfa was fed to the cows at the rate of 1¾ pounds for each 100 pounds of live weight, the silage at the rate of 1½ pounds for each 100 pounds of live weight, and the grain at the rate of 1 pound for each 4 pounds of 4-percent fat-corrected milk. The proper amount of feed for each cow in relation to her weight and production of 4-percent fat-corrected milk was calculated at the beginning of the experiment, and the amount of feed was not altered during the 90 days of the experiment. During the first 20 days of each 30-day period the feeds were weighed to the closest 0.1 pound, and during the last 10 days of each 30-day period the feeds were weighed to the closest gram and the water consumed was weighed to the closest 0.1 pound. The weight of feeds fed per day for the entire 90 days and also the daily weight of dry matter in the orts during the digestion periods are shown in table 2.

At the beginning of each digestion trial, enough grain to last for the full 10 days was thoroughly mixed and sampled, and the 30 feedings for each cow were weighed into paper bags and labeled. The silage was removed from the silo shortly before it was fed and the proper quantity weighed for each cow. Representative samples of the alfalfa, which was fed whole, were secured during each digestion trial. The values for alfalfa shown in table 3 are the averages of these samples. The feeds, orts, and feces were analyzed by the official methods of the Association of Agricultural Chemists (*1*).

TABLE 2.—*Amount of feeds fed per day during entire 90 days, and dry matter in orts during digestion trials, of fumigated-alfalfa experiment*

Cow no.	Feeds fed per day			Dry matter in orts per day		Cow no.	Feeds fed per day			Dry matter in orts per day	
	Alfalfa	Silage	Grain	Fumigated alfalfa	Check alfalfa		Alfalfa	Silage	Grain	Fumigated alfalfa	Check alfalfa
	Grams	*Grams*	*Grams*	*Grams*	*Grams*		*Grams*	*Grams*	*Grams*	*Grams*	*Grams*
16	11,430	9,798	8,301	1,668	450	24	7,893	6,804	5,442	0	0
31	11,160	9,525	4,626	1,768	3,551	32	9,525	8,148	4,083	576	1,477
42	9,117	7,758	5,034	198	494	40	7,893	6,804	5,442	720	108
41	7,893	6,804	4,356	923	331						
18	8,301	7,077	2,859	201	493	Mean				715	845
22	7,077	6,123	3,402	722	910						
45	7,893	6,804	4,899	378	636	S. D					794
						Odds					2.11:1

The percentage of the dry matter in the feeds, and the analyses of the dry matter, are shown in table 3.

TABLE 3.—*Composition of dry matter in feeds fed during digestion trials to cows on fumigated-alfalfa experiment*

Kind of feed and period	Dry matter	Protein	Fat	Fiber	Nitrogen-free extract	Ash
Mixed grain:	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
Period 1	92.39	20.31	9.03	12.34	53.23	5.09
Period 2	91.35	20.83	8.67	14.02	51.39	5.09
Period 3	93.32	19.97	9.57	12.77	52.76	4.93
Silage	22.17	7.18	2.20	29.85	52.21	8.56
Alfalfa, fumigated	87.21	15.29	1.80	32.99	41.54	8.38
Alfalfa, check	85.39	13.45	1.79	36.89	39.88	7.99

Two samples from each of the lots of hay were analyzed for sulphur by the Burgess-Parr bomb method. The average sulphur content on a moisture-free basis for the check hay was 0.384 percent, and for the fumigated alfalfa, 0.617 percent.

The energy of the feeds was determined by the use of the Parr oxygen-bomb calorimeter apparatus. The Benedict oxycalorimeter was used to determine the energy value of the refuse and feces. The energy in the milk was calculated by the method of Gaines and Davidson (*4*).

Sand was used for bedding during the 3 preliminary days of each digestion trial, and canvas mattresses filled with straw were used during the 7 collection days. Attendants were constantly present to collect the excreta. At the end of each 24-hour collection period the excreta were thoroughly mixed and an aliquot portion taken for a composite sample.

Throughout the 90 days of the experiment the cows were weighed between 10 and 11 a. m., and while on digestion trial, at 5 a. m. also. During the digestion period the cows were exercised for 30 minutes by walking 1.25 miles immediately after the 10 a. m. weighing.

The milk was weighed at each milking during the entire 90 days and a 5-day composite sample was secured for butterfat analysis by the Babcock method.

EXPERIMENTAL RESULTS

MILK PRODUCTION

The results secured, as measured by the production of 4-percent-fat-corrected milk while the two hays were consumed, are shown in table 4. The productions during the second 10 days of the first and the third 30-day periods were averaged. This average was compared with the production during the second 10 days of the second 30-day period, in order to compensate for an advancing lactation period. The average production of 4-percent fat-corrected milk per cow while the cows were fed fumigated hay was 345.6 pounds, and while they received the check hay, it was 341.1 pounds. When the data are analyzed by Student's method (*6*), the odds are 2.68:1 that the difference is significant. It is apparent that there was no significant difference in the results secured with the two rations as fed in this experiment.

Data for the milk produced during the rest of the experimental period are not presented in detail since they were concordant with the data presented in table 4. The total production of fat-corrected milk during the portions of the 90-day trial that the 10 cows received fumigated hay was 15,508.3 pounds and during the portions that they received the check hay, 15,151.5 pounds. When the production of the first and the production of the third 30-day period were averaged and compared with the production of the second 30-day period, it was found that 10,276.0 pounds of 4-percent fat-corrected milk was produced while the fumigated hay was fed, and 10,129.2 pounds while the check hay was fed, a difference 0.1 percent greater than that shown by the data in table 4.

TABLE 4.—*Milk production while cows received alfalfa hay fumigated with sulphur dioxide compared with production while cows received nonfumigated hay during 10-day periods of a double-reversal feeding trial*

Cow no.	4-percent fat-corrected milk during fumigated hay periods	4-percent fat-corrected milk during check hay periods	D	D^2	Cow no.	4-percent fat-corrected milk during fumigated hay periods	4-percent fat-corrected milk during check hay periods	D	D^2
	Pounds	*Pounds*				*Pounds*	*Pounds*		
16	603.93	553.76	50.17	2,517.03	45	380.18	369.28	10.90	118.81
31	286.25	310.32	−24.07	579.36	24	377.00	396.38	−19.38	375.58
42	371.88	367.75	4.13	17.06	32	315.02	307.13	7.89	62.25
41	305.08	326.40	−21.32	454.54	40	426.90	407.72	19.18	367.87
18	160.50	167.03	−6.53	42.64					
22	229.55	204.72	24.83	616.53	Total	3,456.29	3,410.49	45.80	5,151.67
					Average	345.63	341.05	4.58	515.167

By Student's method: $S.D. = \sqrt{515.167 - (4.58)^2} = 22.23$

$$\frac{M}{S.D.} = Z = \frac{4.58}{22.23} = 0.21$$

Odds=2.68:1 that there is a significant difference. Odds of 25 or 30 to 1 are required to establish a definite difference.

WEIGHTS

A 3-day running average of the weights of each cow revealed considerable variation in fill and shrink incident to stabling for digestion trials, but there were no significant actual gains or losses in weight

during the 90 days. During the 7-day collection periods, each of the cows was weighed at 5 a. m., the weights at both the beginning and the end of each period being included, which gives a total of eight for each cow for each digestion trial. The average weight of the cows while receiving the fumigated hay was 1,079.8 pounds, and while receiving the check hay, 1,078.2 pounds—a difference of only 1.6 pounds.

PALATABILITY

The rations contained a relatively large quantity of alfalfa and the cows received as much alfalfa as they would consume, or slightly more so that the orts from the two hays might indicate their comparative palatability. In most cases the material refused was alfalfa stems. During the second digestion trial, cow 31 was off feed for a few days and passed a small amount of blood with the urine. This apparently accounts for her high feed refusal during this period.

The dry matter in the orts was determined for the periods that the cows were on digestion trials. The results are shown in table 2. The mean weight of dry matter in the orts while the cows received fumigated hay was 715 g. per day and while they received the check hay, 845 g.

The dry matter in the orts during the first 20 days of each 30-day period was not determined, but the daily weights of orts were practically the same as during the digestion periods.

These results, as well as observations made when rations were changed, indicate no difference in palatability of the two rations.

APPARENT DIGESTIBILITY OF THE COMPLETE RATIONS

The apparent digestibility of the dry matter, energy, protein, ether extract, fiber, nitrogen-free extract, and ash is shown in table 5. In no case was there a significant difference between the two rations.

The correlation (*8*) between the apparent digestibility of the energy and of the dry matter in the 30 trials (0.9441) was very high. The high correlation is clearly indicated in figure 4.

TABLE 5.—*Apparent digestibility of the various constituents of entire rations containing fumigated and nonfumigated alfalfa*

Cow no.	Dry matter		Energy		Protein		Ether extract		Fiber		Nitrogen-free extract		Ash	
	Fumigated	Check	Fumigated	Check	Fumigated	Check	Fumigated	Check	Fumigated	Check	Fumigated	Check	Fumigated	Check
	Per cent	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*
16	64.6	66.6	65.0	67.8	70.6	70.8	89.6	93.0	46.7	52.5	71.7	74.2	46.4	44.1
31	65.7	71.1	65.2	71.3	69.1	75.5	87.2	88.7	52.9	63.6	72.7	75.4	45.6	50.4
42	65.2	65.1	64.0	66.3	70.1	68.9	87.0	87.8	51.2	54.7	72.3	72.3	45.2	38.1
41	65.6	62.8	65.8	63.7	69.6	70.6	86.7	87.7	53.0	51.2	72.5	70.5	42.1	29.4
18	65.1	64.0	64.5	63.8	71.6	70.2	84.6	78.3	54.2	54.4	71.4	71.0	43.8	41.4
22	57.6	60.5	58.8	61.1	67.2	63.1	71.9	84.1	40.9	48.9	67.3	68.7	26.4	32.2
45	65.7	63.3	66.4	63.8	72.5	67.0	88.2	83.1	53.9	49.9	73.4	71.3	29.6	38.2
24	69.3	65.8	69.2	65.4	74.7	69.4	83.6	83.5	55.5	50.5	77.6	74.3	44.3	48.3
32	64.4	67.3	64.2	66.9	72.1	70.7	91.5	80.2	53.1	61.8	70.4	72.4	38.5	44.5
40	67.1	67.0	67.9	67.8	75.8	71.5	90.3	85.9	50.4	57.9	74.8	72.9	39.7	39.4
Mean	65.0	65.3	65.1	65.8	71.3	69.8	86.1	85.2	51.2	54.5	72.4	72.3	40.2	40.6
S. D		3.21		2.87	3.41		6.07			5.25	2.03			6.37
Odds		1.6:1		2.9:1	8.3:1		2.2:1			18.8:1	1.0:1			1.1:1

PERCENT OF ENERGY INTAKE RETURNED IN MILK

The energy in the milk produced by the cows while on the digestion trials was determined by converting the milk production to the equivalent amount of 4-percent fat-corrected milk (*4*). The percentages of energy intake returned in the form of milk when the two rations were fed were determined and compared by Student's method as shown in table 6. The means are 18.65 percent for the fumigated-alfalfa periods and 18.49 percent for the check-alfalfa periods. This difference is not

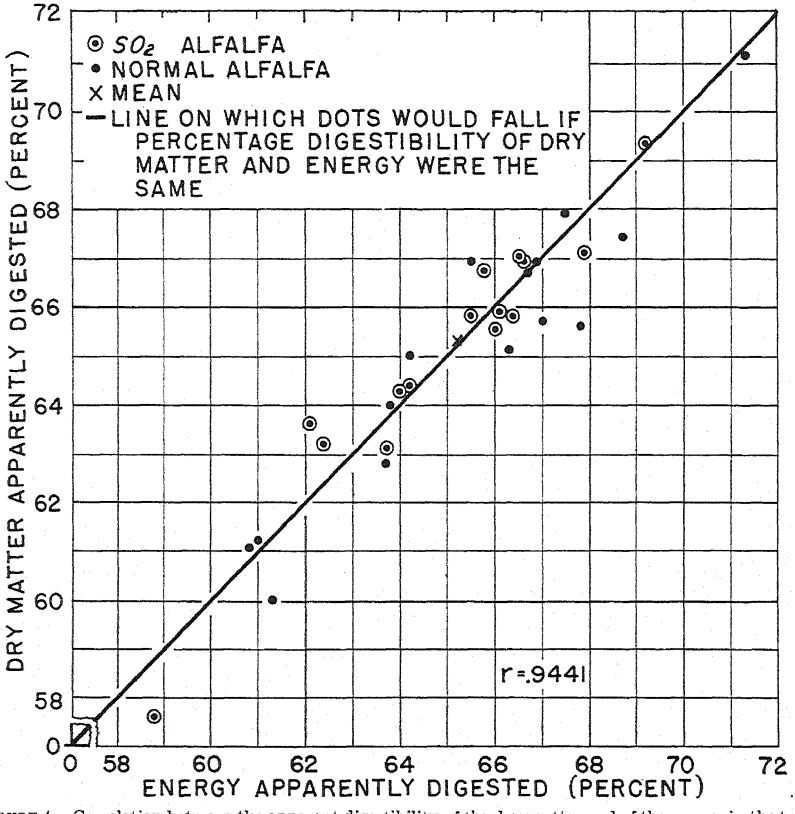


FIGURE 4.—Correlation between the apparent digestibility of the dry matter and of the energy in the total ration. The ration consisted of alfalfa, corn silage, and a grain mixture, and was fed to 10 cows during each of 3 digestion trials.

significant. Forbes and Voris (*3*) show an average return of 20.76 percent of the energy fed to 12 cows throughout the duration of their lactation periods. The variations in their experiments were between 18.04 and 23.35 percent for the individual cows. It may be pointed out that the rations fed to the cows in the experiment of Forbes and Voris were reduced as the lactation advanced to correspond to the production of the individual cows.

TABLE 6.—*Energy intake returned in milk from cows fed rations cotaining fumigated and nonfumigated alfalfa*

Cow no.	Fumigated alfalfa	Check alfalfa	Cow no.	Fumigated alfalfa	Check alfalfa
	Percent	*Percent*		*Percent*	*Percent*
16	22.42	21.87	32	18.78	18.24
31	15.46	18.12	40	23.42	22.58
42	19.37	20.07			
41	17.76	16.33	Mean	18.65	18.49
18	11.59	10.37			
22	15.32	16.57	S. D.	1.2158	
45	22.05	20.83	Odds	1.83:1	
24	20.17	19.95			

pH VALUE OF THE URINE

Every morning while the digestion trials were in progress, a sample of approximately 100 cc of urine was secured from each cow. When the temperature of the samples had reached equilibrium with the temperature of the room (between 60° and 70° F.) in which the potentiometer was located, the pH value was determined. A Leeds and Northrup 7655 potentiometer and a quinhydrone electrode were used. The pH values ranged from 8.22 to 8.43 while the cows were fed the fumigated alfalfa, and from 8.18 to 8.57 while they were fed the check alfalfa. The respective approximate means were 8.30 and 8.38. While the pH value of the urine tended to be lower when the ration contained the alfalfa with the higher quantity of sulphur, the odds of 8.33:1 indicate no significant difference.

SUMMARY

In a 90-day double-reversal feeding trial, 10 purebred cows, Holstein-Friesians, Jerseys, and Guernseys, were fed a ration of mixed grain, corn silage, and alfalfa that had been injured by sulphur dioxide gas. For comparison the same ration was fed, except that nonfumigated alfalfa was used.

The average daily production of 4-percent fat-corrected milk per cow was found to be 34.6 pounds while the cows received the fumigated alfalfa, and 34.1 pounds while they received the check alfalfa during the second 10 days of each 30-day period.

There were no significant changes in the weights of the cows.

The last 10 days of each 30-day period were used to determine the apparent digestibility of the two rations. No significant differences were found in the apparent digestibility of the various constituents of the rations when the results from feeding the two hays were compared. A correlation of 0.9441 was found between the apparent digestibility of the dry matter and the apparent digestibility of the energy.

During the digestion trials the average daily refusal of dry matter was 715 and 845 g per cow while receiving the fumigated alfalfa and the check alfalfa, respectively. The difference is not significant.

Of the ingested energy, 18.65 percent was returned in milk while the cows were receiving the fumigated alfalfa, and 18.49 percent while they were receiving the check alfalfa. The difference is not significant.

While the pH value of the urine voided by the cows when receiving the fumigated alfalfa was slightly lower than when receiving the check alfalfa with its somewhat lower sulphur content, the difference is not significant.

CONCLUSION

No significant increase or decrease in the feeding value of alfalfa hay made from alfalfa subjected to a sufficient amount of sulphur dioxide to cause acute injury to more than 25 percent of the leaflets was apparent under the conditions of this experiment.

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CERTAIN RELATIONSHIPS BETWEEN THE CALCIUM AND OXALATE CONTENT OF FOLIAGE OF CERTAIN FOREST TREES [1]

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INTRODUCTION

That calcium oxalate crystals exist in the tissues of many plants is a well-established fact. Little investigation, however, has been conducted to determine what proportion of the calcium is present as the oxalate, or what percentage of the oxalate ions are combined with calcium. This paper shows in what form the oxalate ions exist in the leaves of certain forest trees and indicates certain associations between the calcium content and the oxalate content of the leaves.

No attempt is made at this time to formulate a theory which associates the calcium requirements of forest trees with the production of oxalic acid. Such a relationship may exist, but the data included in this paper do not adequately substantiate such a theory. Further studies are being conducted on this problem.

EXPERIMENTAL METHODS

The trees from which the leaf samples were picked were growing in the vicinity of Ithaca, N. Y., on Dunkirk silty clay loam and Lordstown stony silt loam. The Dunkirk soil is derived from glacial lake-laid deposits, and has an acid surface soil, but a calcareous subsoil. The Lordstown soil is derived from glacial till composed of fragments of shale and sandstone. It is acid throughout the profile.

The leaf samples were secured between September 10 and 15, 1936, according to the technique described by Mitchell.[2] The samples were dried for 24 hours at a temperature of 70° C., and subsequently ground so that all of the material passed through a 40-mesh sieve.

In the determination of total calcium, a ½-g sample was ignited in an electric muffle furnace at a temperature of approximately 800° C. The ash was taken up with hydrochloric acid and transferred quantitatively to a 150 ml beaker. The calcium was precipitated as the oxalate by the usual method, and titrated with 0.05 normal potassium permanganate solution.

The total oxalate content of the tissues was determined by the ether extraction method of Pucher, Vickery, and Wakeman.[3]

The acetic acid-insoluble calcium was determined as follows: A 25-ml portion of normal acetic acid solution was added to a 1-g leaf

[1] Received for publication June 17, 1937; issued September 1937.

[2] MITCHELL, H. L. TRENDS IN THE NITROGEN, PHOSPHORUS, AND POTASSIUM CONTENT OF THE LEAVES OF SOME FOREST TREES DURING THE GROWING SEASON. Black Rock Forest Papers 1, no. 6, illus. 1936.

[3] PUCHER, G. W., VICKERY, H. B., and WAKEMAN, A. J. DETERMINATION OF THE ACIDS OF PLANT TISSUE. II. TOTAL ORGANIC ACIDS OF TOBACCO LEAF. Indus. and Engin. Chem., Analyt. Ed. 6: 140-143. 1934.

sample and the mixture was agitated on a mechanical shaker for 10 minutes and then filtered on asbestos by the use of suction. It was next washed with successive 25-ml portions of the acetic acid solution until somewhat more than 200 ml had passed through the filter. The filtrate was then transferred to a 250 ml volumetric flask. A 50-ml aliquot was evaporated to dryness and the calcium content determined in the same manner as described above. This value, the acetic acid-soluble calcium, was subtracted from the total calcium content to determine the amount which was insoluble in acetic acid. It was assumed that this fraction corresponded to calcium in the form of calcium oxalate.

EXPERIMENTAL RESULTS

The total calcium content, total oxalate content, and acetic acid-insoluble calcium of the foliage of various tree species are presented in table 1. The results are expressed as milliequivalents per 10 g of oven-dried material. The percentage of calcium insoluble in acetic acid and the series designation of the soils upon which the trees were growing are also reported. The tree species are listed in the order of their increasing total calcium content.

TABLE 1.—*Total calcium content, total oxalate content, and acetic acid-insoluble calcium content of foliage of various forest trees*

Tree species	Soil series	Total calcium per 10 g	Total oxalates per 10 g	Calcium insoluble in acetic acid per 10 g	Portion of total calcium insoluble in acetic acid
		Milli-equivalents	*Milli-equivalents*	*Milli-equivalents*	*Percent*
White pine, *Pinus strobus* L. (new needles)	Dunkirk	2.65	1.55	1.53	57.7
Red oak, *Quercus borealis* var. *maxima* (Marsh.) Ashe	Lordstown	5.20	2.47	2.45	47.2
Chestnut oak, *Quercus montana* Willd	do	6.00	3.21	3.25	54.2
Yellow birch, *Betula lutea* Michx	do	7.51	4.45	4.50	59.9
Sugar maple, *Acer saccharum* Marsh	do	7.95	4.75	4.70	59.1
White cedar, *Thuja occidentalis* L. (new leaves)	Dunkirk	8.75	6.01	5.99	68.4
Red cedar, *Juniperus virginiana* L. (new leaves)	do	8.85	6.55	6.60	74.6
White ash, *Fraxinus americana* L	Lordstown	10.05	5.51	5.65	56.2
White cedar (old leaves)	Dunkirk	11.30	8.54	8.55	75.6
Ironwood, *Ostrya virginiana* (Mill.) Koch	Lordstown	13.10	8.51	8.60	65.6
Trembling aspen, *Populus tremuloides* Michx	do	13.24	5.71	5.52	41.6
Do	Dunkirk	13.30	6.41	6.43	48.3
Pignut hickory, *Hicoria glabra* (Mill.) Sweet	Lordstown	13.31	12.69	11.52	86.6
Red cedar (old leaves)	Dunkirk	14.81	13.62	11.81	79.7
Pignut hickory	Lordstown	16.85	16.12	14.98	88.9
Basswood, *Tilia americana* L	do	18.13	10.91	10.92	60.2

There was a distinct tendency for the total oxalates to be correlated with the total calcium content. Trembling aspen and basswood were outstanding exceptions to this rule, and white ash and ironwood were somewhat out of line. The total oxalates did not exceed the total calcium in any case.

A comparison of the total oxalates and acetic acid-insoluble calcium shows a close agreement in nearly all cases. This seems to support the conclusion that in most cases all of the oxalate ions were combined with calcium. Pignut hickory and red cedar were the only two exceptions to this rule.

An examination of the last column of table 1 shows that the percentage of the total calcium present as calcium oxalate varied con-

siderably. The two aspens and red oak were lowest in this respect. The white cedar, red cedar, and pignut hickory were highest. There was a close agreement between the figures for the two aspen samples, as well as for the two hickory samples, although each tree was growing on a different site. The data also indicate that when the calcium oxalate content reaches a certain value (79 percent or above) in relation to the total calcium any oxalic acid formed in excess of that amount will be in some form other than calcium oxalate.

DISCUSSION

The fact that the calcium content always exceeded an equivalent amount of oxalates is of particular interest. Of course, if all the oxalates were present as calcium oxalate, the above situation would have to exist. The question arises, however, as to why the production of oxalic acid in the leaves was maintained within such limits as to prevent it from exceeding the calcium content, or, conversely, why the absorption of calcium was limited to such an extent that it did not exceed the oxalate content to any great degree. Parker and Truog [4] advanced the theory that the amounts of calcium and nitrogen in plant tissues are highly correlated because of the fact that protein metabolism is one of the chief sources of organic acids and the calcium absorbed would function in the neutralization of these acids.

The total nitrogen content of the leaves used in this study was determined. There was essentially no relationship between the nitrogen and the oxalate content. For example, the old red cedar leaves contained only 1.34 percent of nitrogen, yet the oxalate content was 13.62 milliequivalents. Red oak, on the other hand, had a nitrogen content of 2.05 percent, with an oxalate content of only 2.47 milliequivalents. It would appear, therefore, that the production of oxalic acid in the forest tree leaves used in this study was dependent upon more factors than simply the nitrogen content. Furthermore, Dunne [5] has indicated that calcium does not necessarily function in the neutralization of organic acids, since other bases such as potassium are sufficient for the process.

Another factor which complicates the explanation of these results is that two species, namely, cucumbertree, *Magnolia acuminata* L., and tuliptree, *Liriodendron tulipifera* L., did not contain any oxalates. They had a calcium content of 7.70 and 15.15 milliequivalents, respectively. All of the calcium in each case was soluble in normal acetic acid solution. It is of interest to note that cucumbertree and tuliptree belong to the same botanical family, which further indicates that the production of oxalic acid by plants may be an inherent characteristic, and is not necessarily influenced by environment.

If the absorption of calcium is strongly influenced by the precipitation of calcium as calcium oxalate within the plant, one would expect those species which produce no oxalic acid to contain relatively small amounts of calcium. But, as has been shown above, such may not be the case. There are other forms of water insoluble calcium, of course, which would be soluble in acetic acid and would not be revealed in this study. Such forms might be more abundant in those

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species which had a high calcium content with small amounts of oxalates or none. Additional study is needed on this point.

The fact that, with the exception of two species, essentially all of the oxalates could be accounted for as calcium oxalate is interesting. This is not true of certain other plants. Lebedeva and Pochinok,[6] working with sugar-beet leaves, showed that water-soluble oxalates were present in quantities up to five times the amount of calcium oxalate, and, moreover, they found no water-soluble or acetic acid-soluble calcium. Dunne has shown that buckwheat plants may contain in equivalent amounts nearly twice as much oxalate as calcium. His data indicated that oxalic acid could be precipitated in a relatively water-insoluble form by either calcium or potassium, the latter case resulting in the formation of potassium acid oxalate.

The fact that the calcium content of the older leaf tissues, in the case of the cedars, was greater than the oxalate content, even though the oxalate content had increased over that of the younger tissues, again showed a distinct relationship between calcium and oxalates. Whether the calcium content was related to the oxalate content or whether outside factors influenced both values, is a matter for conjecture. It does not seem likely however, that these relationships were entirely accidental.

Further work is needed on the forms of water-insoluble calcium, on the metabolic availability of calcium as calcium oxalate, and upon the actual calcium requirements of forest tree species.

SUMMARY

A study has been made of the relationship between the calcium and the oxalate content of the foliage of various forest trees.

The total oxalates in the leaves were, in general, found to be correlated with the total calcium content.

In none of the species studied did the total oxalates exceed the total calcium content.

Except in two species, all of the oxalates were present as calcium oxalate.

The leaves of certain forest trees may not contain any oxalates, yet the calcium content may be high.

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POTENTIALITIES OF ERADICANT FUNGICIDES FOR COMBATING APPLE SCAB AND SOME OTHER PLANT DISEASES[1]

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In a recent paper, one of the writers (*15*)[3] called attention to the current reliance on protectant spraying as the dominant measure for control of many fruit diseases, and suggested the desirability of increased effort to develop control programs of complementary procedures based on the principles of immunization and eradication, as well as protection. The purpose of the present paper is to report the progress of studies of potentialities for increased application of the principle of eradication to the control of certain types of plant disease through the use of eradicant[4] fungicides. The experimental basis of several brief preliminary reports on this work (*13, 14, 15, 16, 17, 18, 19, 20, 25*) is recorded herein.

STUDIES ON APPLE SCAB

INTRODUCTION

Apple (*Malus sylvestris* Mill.) scab, caused by *Venturia inaequalis* (Cke.) Wint., is the most widespread and important disease of deciduous orchard fruits. Despite the excellent progress that has been made in developing measures for its control, it continues to take a heavy toll from the growers and consumers of apples.

Since the advent of bordeaux mixture, protectant spraying (or dusting) has been the dominant measure for apple scab control. So great has been its success that comparatively little attention has been given to the development of complementary measures.

Failures of approved spraying practices to give adequate scab control in Wisconsin led one of the writers and his associates (*10, 16, 24, 33*) to undertake a series of studies relating to the epidemiology and control of this disease. The earlier work (*16*) indicated (1) that, under the conditions encountered in this State, ascospores comprise the only important primary inoculum, and (2) that the quantitative level of the ascosporic inoculum is of prime importance in relation to epidemiology and control. It was further shown that protectant spraying is adapted primarily to control of the disease on the fruit, and ordinarily fails to control leaf infection sufficiently to preclude the development of a dangerous ascosporic inoculum in the following spring. Since all the blossoms expand in a relatively short time, it is usually feasible to keep them and the developing fruits

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[3] Reference is made by number (italic) to Literature Cited, p. 435.

[4] It is obvious that some eradicant fungicides may have protectant value, and vice versa. In this paper fungicides are referred to as eradicant or protectant according to their dominant role.

adequately protected by fungicidal treatments. However, new leaves are produced during a much longer period, and unprotected leaf surface may be exposed between fungicidal treatments of any feasible frequency. The current method of scab control by protectant fungicides is, therefore, based chiefly on a philosophy of defense. The pathogen is permitted to maintain a high survival level, and under conditions of moderate or severe occurrence of the disease there is usually little or no cumulative control (*15*, *16*).

These considerations suggested a critical reexamination of the current defensive methods of apple scab control and an exploration of possibilities for developing complementary offensive measures designed for direct attack on the pathogen, with the aim of reducing it to a quantitative level at which it might be more surely and economically controlled.

Despite its great accomplishments, the protectant fungicidal method of scab control has some sharp limitations. The most fundamental one, its failure to reduce the pathogen to a sufficiently low survival level to accomplish satisfactory cumulative control, has just been discussed. A further and increasing difficulty in the protectant program is the conflict between the requirements of sufficient toxicity for adequate control of the disease and freedom from objectionable host injury. Increasing age of orchards and concentration of apple culture tend to increase the severity of the disease and, consequently, the need for protection against the fungus. At the same time, rising standards of quality of fruit and increased knowledge of effects of host injury make for lessening the tolerance of toxicity to the host. Considerations of host injury have led to the use of progressively weaker fungicides. Bordeaux mixture has largely been replaced by lime-sulphur, which in turn is being replaced by less toxic materials. However, decreased toxicity of the fungicide necessitates increased frequency and cost of application or increased danger of failure to control the disease. Another limitation of programs based too exclusively on the use of protectant fungicides is the constant danger of serious failure to control the disease in the event of unusually severe epidemics or unavoidable dislocations in the program of applications incident to weather conditions, failure of machinery, or other causes. Furthermore, these programs are objectionably laborious, time consuming, and expensive.

In exploring the possibilities of developing control measures to complement protectant spraying, attention was turned first to consideration of means for a direct attack on the pathogen, with the immediate objective of reducing its survival level sufficiently to facilitate control. It was thought that a sufficiently drastic reduction in the ascosporic inoculum might insure the success of the protectant program, even under the most favorable conditions for scab development, and that lessening the severity of the strain on protectant spraying might permit the use of milder fungicides or fewer applications. The nearest feasible approach to local eradication of the pathogen was regarded as the ultimate objective of this line of work.

There have been many previous attempts to apply eradicant measures to apple scab control; but, whether because the idea is impractical or the methods developed were not sufficiently effective, comparatively little progress has been made.

Disposal of the fallen leaves by burning or burying was empirically recommended long before it was proved that the scab fungus over-

winters in them. Turning the leaves under by clean culture before the ascospores are discharged is often recommended, and is generally regarded as a desirable aid to scab control in situations where such cultivation is a feasible and economic horticultural practice. However, there are a great many situations in which this procedure is not feasible; and, furthermore, exact studies of its effectiveness in relation to the epidemiology and control of scab are lacking. The chief limitation to its success in situations where it can be practiced advantageously appears to be its comparative inefficiency as an eradicant measure.

Possibilities of reducing the survival level of the scab pathogen by spraying have received consideration from time to time; but advances in this direction have been sharply limited both by insufficient understanding of basic phenomena in the epidemiology and control of the disease and lack of suitably adapted materials and methods. Experimentation in this direction has hitherto been dominantly concerned with fungicides that were developed primarily for protectant spraying. For reasons that have been stated, efforts to suppress the pathogen through cumulative effects of protectant spraying have met with comparatively little success. Jehle and Cory (*12*), following a suggestion of W. C. Travers, tested the value of an application of bordeaux, 10–10–50, made just after harvest and before many of the leaves had fallen, thinking that it might check spore production in the fallen leaves in the spring. They concluded that, "When no other material was applied except Bordeaux in the fall, there was apparently a slight gain in the number of fruits free from scab, but this increase was so slight that as an added treatment Bordeaux would not be profitable". Curtis (*4*), tested the effects of spraying the leaves on the ground in the spring with lime-sulphur at the strength used on trees in foliage. Three such applications of spray caused a definite reduction in the incidence of foliage infection, whereas a single treatment did not. She states that, "Application of the spray three or four days before rain does not necessarily prevent the discharge of living ascospores; maximum effectiveness of the spray is secured only when it is applied *immediately after rain*". Early in the development of spraying methods for apple scab control, a dormant application of bordeaux was sometimes recommended as a "clean-up" spray; but the results did not justify general adoption of this practice. In situations where the scab pathogen overwinters in twig lesions or bud scales, dormant applications of lime-sulphur or bordeaux are sometimes employed, though fungicides better adapted to the purpose would seem desirable.

During the course of the work reported in the present paper (cf. *16*, *19*, *20*), Folsom and Ayers (*6*) found that an application of copper sulphate solution, 5–50, after harvest apparently caused no reduction in the development of ascocarps of the scab fungus in the following spring. Wiesmann (*31*) states that, in similar small-scale experiments in one season, each "1% Helion-Winter, Ciba 1930" and "8% Obstbaumkarbolineum Maag" hindered or prevented the formation of perithecia, whereas "20% Schwefelkalkbrühe Siegfried" did not. He reports further that treating the leaves with "5% Obstbaumkarbolineum" as they lay on the ground under the trees in the spring killed 85.6 percent of the ripe perithecia.

In the present investigation eradicant chemical treatments have been directed against the pathogen at two seemingly vulnerable stages

in its life history. The first of these is the period after harvest but before many leaves have fallen. At this time the leaves can still be thoroughly covered by spray and the trees will tolerate a much more drastic treatment than is feasible earlier in the growing season. The other vulnerable stage is after leaf fall but before ascospores are discharged. Still more drastic treatments can then be given to the leaves on the ground. The work on fall spraying is reported first.

THE EARLIER EXPERIMENTS ON FALL SPRAYING, 1924–31

Studies of the possible adaptation of a fall application of eradicant fungicides to apple scab control were started in 1924, and have been continued in each succeeding year. Since the chief purpose of the earlier work (1924–31) was to narrow the problem, it will not be reported in detail. A brief account of it follows.

Branches or whole trees bearing diseased leaves were sprayed after harvest and before many leaves had fallen, and representative samples of sprayed and unsprayed leaves were picked, shortly before they would have fallen, and overwintered on sod in cloth net bags. In the following spring, by means of microscopic examination, records were made on the occurrence of perithecia of *Venturia inaequalis*.

The calcium, copper, and lead arsenites used prior to 1930 and the magnesium arsenite were prepared in the laboratory. The mono- and dicalcium arsenites and the dicalcium arsenate used in 1930, which were not free from admixtures of homologous calcium arsenites or arsenates, respectively, were obtained from manufacturers. The other materials used were obtained from commerical sources.

Experiments of 1924.—A great reduction in the number of perithecia followed the use of each copper sulphate, 1 percent; sodium nitrate, 15 percent; neutral bordeaux (using 1 percent $CuSO_4.5H_2O$) plus sodium aresnite, ¼, ½, and 1 percent, respectively; and certain highly alkaline copper sulphate-potassium hydroxide mixtures. Abundant perithecia developed in the unsprayed leaves and those that had been sprayed with each bordeaux mixture, 6–6–50; lime-sulphur, 1–10; 1 mixture of bordeaux and lime-sulphur, each at the strength just stated; bordeaux, 6–6–50, plus a proprietary sodium polysulphide spray at the strength recommended for dormant spraying; neutral bordeaux (using 1½ percent $CuSO_4.5H_2O$) plus mercuric chloride, 1–1,000; bordeaux 6–6–50, plus certain additions of sodium or potassium hydroxide; neutral bordeaux (using 1½ percent $CuSO_4.5H_2O$) plus a proprietary casein-lime spray supplement; and the last mentioned mixture plus copper sulphate, three-fourths of 1 percent. Severe host injury resulted from many of these sprays, especially copper sulphate and the preparations containing sodium arsenite.

Experiments of 1925.—Comparatively high effectiveness against the fungus was shown by each sodium arsenite, one-eighth of 1 percent; paris green, 1 percent; these two materials at the same rates, used together; bordeaux, 4–4–50, plus paris green, 1 percent; neutral bordeaux (using 1 percent $CuSO_4.5H_2O$) plus calcium arsenite, 1 percent; calcium arsenite, one-half of 1 percent; and calcium arsenite, 1 percent, plus sodium arsenite, one-fourth of 1 percent. In many cases no perithecia developed in the treated leaves. They developed fairly abundantly in the untreated leaves. Most of the treatments were seriously injurious to the host.

Experiments in 1926.—Several mixtures of calcium arsenite, ½ or 1 percent, and neutral bordeaux (using 1 percent $CuSO_4.5H_2O$) or lime, one-half of 1 percent, greatly reduced the number of perithecia without drastic injury to the host. Similar mixtures in which paris green replaced the calcium arsenite gave less control and more host injury. Sodium and calcium silicofluoride, respectively, 1 or 2 percent, occasioned little reduction in the number of perithecia. Perithecia developed abundantly in the untreated leaves.

Experiments in 1927.—Various mixtures of calcium arsenite with bordeaux, or with lime plus tricalcium phosphate (the latter added because of its possible influence on reaction of the spray residues after weathering), were highly effective in preventing the production of perithecia, some of them causing little host injury. Similar mixtures with paris green as the arsenite were less effective. Zinc arsenite was comparatively ineffective, whether used alone or in mixtures with acetic acid or tricalcium phosphate. Pine oil in concentrations up to 10 percent was ineffective. Perithecia developed abundantly in the untreated leaves.

Experiments in 1928.—Calcium, zinc, copper, magnesium, and lead arsenites, respectively, were ineffective in suppressing the perithecia at concentrations tolerated by the host. Bordeaux, 3–2–50, plus calcium arsenite, 1 percent, reduced the number of perithecia by 96 percent. The same reduction was effected by a mixture of calcium arsenite, 1 percent; lime one-fourth of 1 percent; tricalcium phosphate, one-fourth of 1 percent; and a proprietary casein-lime spray supplement, one-sixteenth of 1 percent. Perithecia developed abundantly in the untreated leaves.

Experiments in 1929.—Numerous mixtures of bordeaux, 3–2–50, and calcium arsenite completely suppressed the development of perithecia. When the calcium arsenite component was one-fourth of one-half of 1 percent, the number of perithecia was reduced by from 91 to 99.8 percent, without severe host injury. Certain mixtures of bordeaux and copper arsenite gave very similar results. A casein-lime spray supplement did not significantly improve the effectiveness of the copper-lime-arsenic mixtures, but a summer spray miscible oil showed some promise as a supplement. Perithecia developed abundantly in the untreated leaves.

Experiments in 1930.—A summer spray miscible oil, 1 percent, was used as a supplement to all the materials tested this year. Tri- and dicalcium arsenate, each used alone or mixed with bordeaux, 3–2–50, were comparatively ineffective. Lead, zinc, and copper arsenites and paris green, each used alone in concentrations of ½ or 1 percent, gave unsatisfactory results. If the perithecia were efficiently suppressed, there was too much host injury. In mixtures with bordeaux, 3–2–50, dicalcium arsenite seemed slightly less effective than monocalcium arsenite. Such mixtures as copper sulphate, three-fourths of 1 percent, lime, one-half of 1 percent, and monocalcium arsenite, one-half of 1 percent, gave good suppression of the perithecia, without severe host injury. Similar results were obtained by reducing the monocalcium arsenite to one-fourth of 1 percent, and adding the less soluble zinc or tricalcium arsenite, 1 percent. The development of perithecia in the untreated leaves was less abundant than usual.

Discussion.—The earlier experiments showed that the commonly used protectant fungicides that were tried have little value for the

purpose in view. Various chemical agents known to be toxic to fungi or other plants were then tested. Arsenites were found to be highly effective in suppressing the perithecia; but they were objectionably injurious to the host and had unsatisfactory physical properties when used alone or with the spray supplements tried. Mixtures of certain arsenites and bordeaux were the most promising materials tested. They were found to be highly effective against the fungus, good in physical properties, less injurious to the host than arsenites used alone at effective concentrations, and comparatively cheap. Arsenites of a wide range of solubility are available, and it was early apparent that the toxic properties of copper sulphate-lime-arsenite mixtures could be varied through a wide range by suitable choice of the arsenite and modifications in the concentrations and proportions of the ingredients.

FURTHER EXPERIMENTS ON FALL SPRAYING, 1931–36

The earlier work having demonstrated that a fall application of a suitable spray may be highly effective in preventing the development of perithecia of the scab pathogen, attention was next directed to a more intensive study of the comparative values of some of the more promising materials.

COMPARISON OF FUNGICIDAL MATERIALS

MATERIALS AND METHODS

Arsenical compounds.—All the arsenical compounds used, which were obtained from commercial companies, were finely ground, usually being capable of passage through a 300-mesh sieve. Results of analyses [5] made according to the methods of the Association of Official Agricultural Chemists (*1*) are shown in table 1, with records of the years in which the several compounds were used and the symbols by which they are designated, for brevity, in the spray formulas. The percent of moisture is based on the original air-dry samples; that of each water-soluble and total arsenic, on the oven-dry (110° C.) samples.

TABLE 1.—*Moisture, water-soluble arsenic, and total arsenic content of arsenical compounds used in the experiments of 1931–36*

Material	Years used	Symbol [1]	Moisture	Water soluble arsenic (As_2O_3)	Total arsenic (As_2O_3)
			Percent	*Percent*	*Percent*
Monocalcium arsenite	1931	Cai			
Do	1932	Cai	0.4	24.46	72.86
Do	1933	Cai	.2	21.15	74.97
Do	1934–35	Cai	.9	23.11	74.59
Dicalcium arsenite	1931–34	2 Cai	4.5	14.86	60.61
Tricalcium arsenite	1931	3 Cai			
Do	1932–33	3 Cai	1.0	1.69	55.06
Do	1934	3 Cai	7.0	5.09	31.45
Zinc arsenite	1932	Zai	.1	.29	40.68
Do	1933–35	Zai	.4	.59	41.73
Zinc arsenite, buffered	1935	B Zai	.1	.06	29.75
Copper arsenite	1931	Copai			
Do	1933	Copai	1.3	1.04	38.88
Do	1934	Copai	2.2	2.02	40.59
Do	1935	Copai	4.3	.96	27.96
Paris green	1931–32	P G	.3	.15	57.48
Iron arsenite	1933	Iai	4.2	2.42	39.22
Do	1934	Iai	5.6	2.99	41.60
Lead arsenite	1933–34	Lai	.3	2.93	42.71
Magnesium arsenite	1933–34	Mai	4.2	42.35	74.47
Dicalcium arsenate	1931	2 Caa			
Do	1932	2 Caa			
Do	1933	2 Caa	.6	[2] 12.83	[2] 50.61
Tricalcium arsenate	1931	3 Caa			

[1] For the sake of brevity these symbols are used in the spray formulas. [2] Expressed as As_2O_5.

[5] These determinations were made by C. N. Clayton under the supervision of Prof. V. W. Meloche.

Copper sulphate.—Technical copper sulphate crystals.

Lime.—A lump lime containing not less than 99 percent of calcium oxide.

Fish oil.—Cold-pressed menhaden fish oil.

Casein-lime.—A commercial spray supplement.

Summer spray miscible oil.—A commercial product.

Experimental trees.—For the applications of 1931–33, trees of either Plumb Cider or an unknown variety, about 20 years old, of low to moderate vigor and in sod, were used. For the later work, Dudley trees in a vigorous young orchard, planted in 1931 and cultivated each year, were employed. Summer spraying of these trees was omitted, in order that the leaves might be severely scabbed. The work was centered mainly on the Plumb Cider and Dudley varieties, because the previous experience had shown these to be the most difficult, of the varieties tested, on which to suppress perithecial development. The experimental conditions for suppression of the fungus were, therefore, much more severe than would ordinarily be encountered in commercial orchards.

Mixing the sprays.—The required amount of a 10-percent aqueous solution of copper sulphate was placed in a mixing pail containing about three-fourths of the total volume of water, and milk of lime (containing 10 percent CaO) was added. The arsenical compounds, which had been made into a thin paste with water, were then added, followed by the spray supplement, if any, and the remainder of the water. The mixtures were vigorously agitated immediately after each addition of material, and applied promptly.

Applying the sprays.—The sprays were applied to selected branches by means of 6- to 8-liter hand sprayers. Care was taken to cover all the leaves as thoroughly as feasible on both sides and to avoid drift of sprays to other experimental branches. Nearly all applications were made during the first week of October. The treatments were made on dry foliage and under conditions that permitted the spray to dry before rain occurred, as slow drying tends to increase host injury by copper-lime-arsenic mixtures.

Overwintering the leaves.—In all the experiments reported in tables 2 and 3, the leaves were overwintered in cloth mesh bags (fig. 1, *A*), laid on sod in the orchard and fastened at the corners with large nails driven into the soil. Ordinarily from 30 to 50 leaves that showed abundant scab infection on the dorsal surface were placed in each bag, and in many cases the samples were laid down in duplicate or triplicate. The leaves were not allowed to overlap each other, and all were exposed with the dorsal surface up. In nature, the perithecia tend to be borne more abundantly on the dorsal surface. An examination of overwintered Dudley leaves under natural conditions in the spring showed that about 75 percent lay with the dorsal surface up. The overwintered experimental samples were allowed to remain in the field in the spring as long as was possible without undue deterioration of leaves or ascocarps. They were then stored dry in the laboratory. In the dry springs of 1934 and 1936, in order to give the ascocarps more favorable opportunity for development, all the samples were moistened with distilled water when they were brought from the field, and held in a cool chamber for several days.

In 1934–35, in order to determine whether substantially different results might follow if the treated leaves were overwintered with the

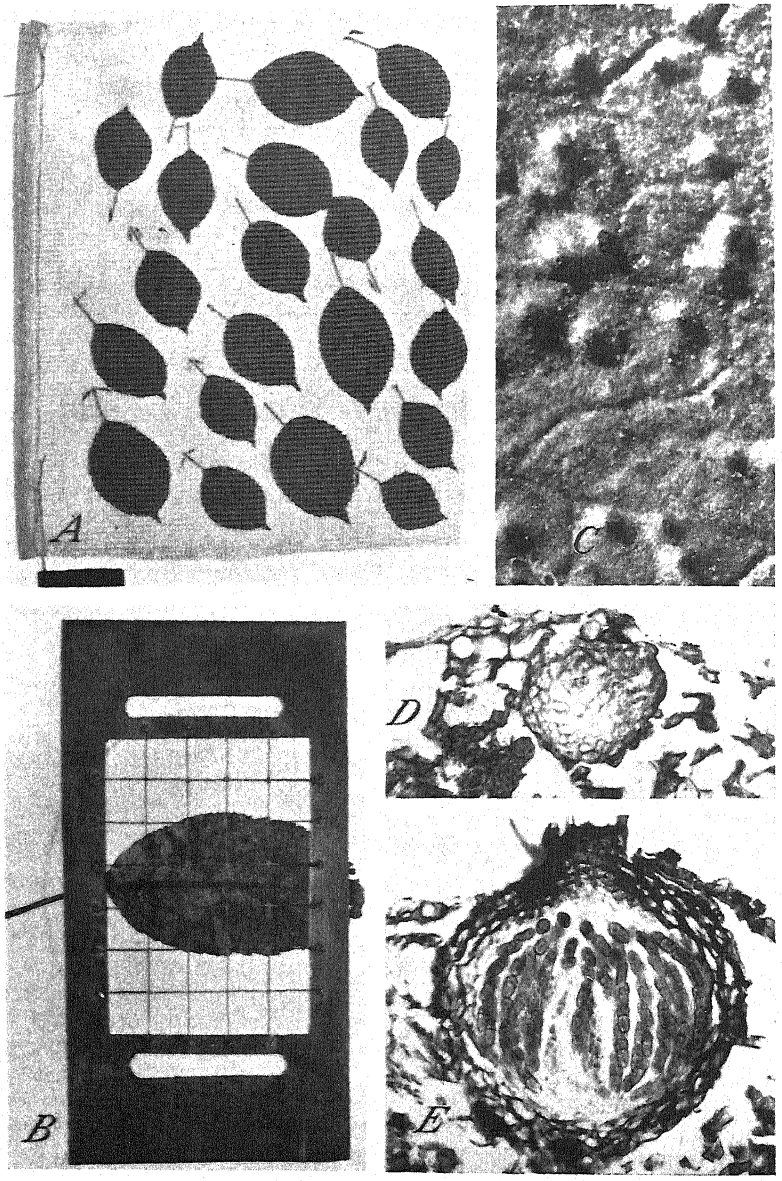


FIGURE 1.—*A*, Apple leaves in open-mesh cloth bag ready for overwintering; *B*, leaf and measuring device in position for counts of perithecia; *C*, apple leaf showing perithecia of *Venturia inaequalis* at the magnification (× 20) at which the counts were made; *D*, section of a treated leaf (formula 139 of table 2) showing a disorganized ascocarp initial, × 425; *E*, section of an untreated leaf (control on *D*) showing a normal perithecium, × 425.

ventral surface up, samples from 26 different treatments were placed on the sod in duplicate with the dorsal surface up in one series and the ventral in the other. The results of the two series were closely similar.

In 1935–36 the development of perithecia in leaves overwintered by the standard method was compared with that in similar leaves overwintered in bags laid on the surface of freshly cultivated soil. In both the treated and untreated samples, somewhat more perithecia were produced in the leaves that lay on the soil than in those on the sod. The spring of 1936 being comparatively dry, the greater moisture accessible to leaves on the soil was beneficial to the fungus. In wet years, however, many leaves thus exposed would disintegrate before the ascospores were discharged.

Counting the perithecia.—Results were usually taken on 20 to 30 leaves per sample. Occasionally disintegration necessitated using a smaller number. After having been soaked in water for a few minutes, the leaf was appressed, weathered surface up, to a small plate of glass and the surplus water absorbed by a wad of cheesecloth. Under the dissecting microscope counts were made of the visible perithecia in ¾-inch areas of leaf surface that bore them in greatest number. Crossed wires held in a rectangular brass frame that was laid on the leaf made it possible to measure the desired areas conveniently without duplication (fig. 1, *B*). Perithecia at the magnification at which the counts were made are shown in figure 1, *C*. Identification of the perithecia was facilitated by pricking out doubtful bodies with a dissecting needle, and examining them under higher magnification if necessary. A disorganized ascocarp initial in a section of a treated leaf is illustrated in figure 1, *D*. Such bodies are not visible from the surface, and are not included in the counts. Figure 1, *E*, illustrates a normal perithecium in a section of an untreated leaf. Most of the samples were examined by more than one worker and without knowledge of the treatment. The results of the counts are expressed as the average number of perithecia per square inch of leaf surface on which the counts were made.

When perithecia occurred in leaves that had received treatments of comparatively high efficiency, they were commonly found in localized areas small enough to be fully included in the counts. The counted perithecia of the controls, however, ordinarily included only a small percentage of the entire number visible on the leaf. Comparisons based on the total number for the entire leaf area, therefore, would indicate a substantially higher degree of effectiveness for the more efficient treatments than is shown by the method used.

ENVIRONMENTAL CONDITIONS

The development of perithecia is greatly influenced by environmental conditions, especially moisture and temperature (*33*). Figure 2 shows daily rainfall and maximum and minimum temperatures recorded by the Madison station of the United States Weather Bureau from September 19 to May 21, in the years 1931–36.

Though temperature is a cardinal factor conditioning perithecial development, it is not ordinarily a sharply limiting one in Wisconsin. Moisture relations, however, are of prime importance in determining the quantity of perithecia produced. Furthermore, they exert an important influence on the effectiveness of the eradicant fungicidal treatments. Enough moisture is essential to dissolve the materials employed sufficiently for them to exercise their toxic effects. However too high rate of loss of the spray residues limits the effectiveness of the treatments.

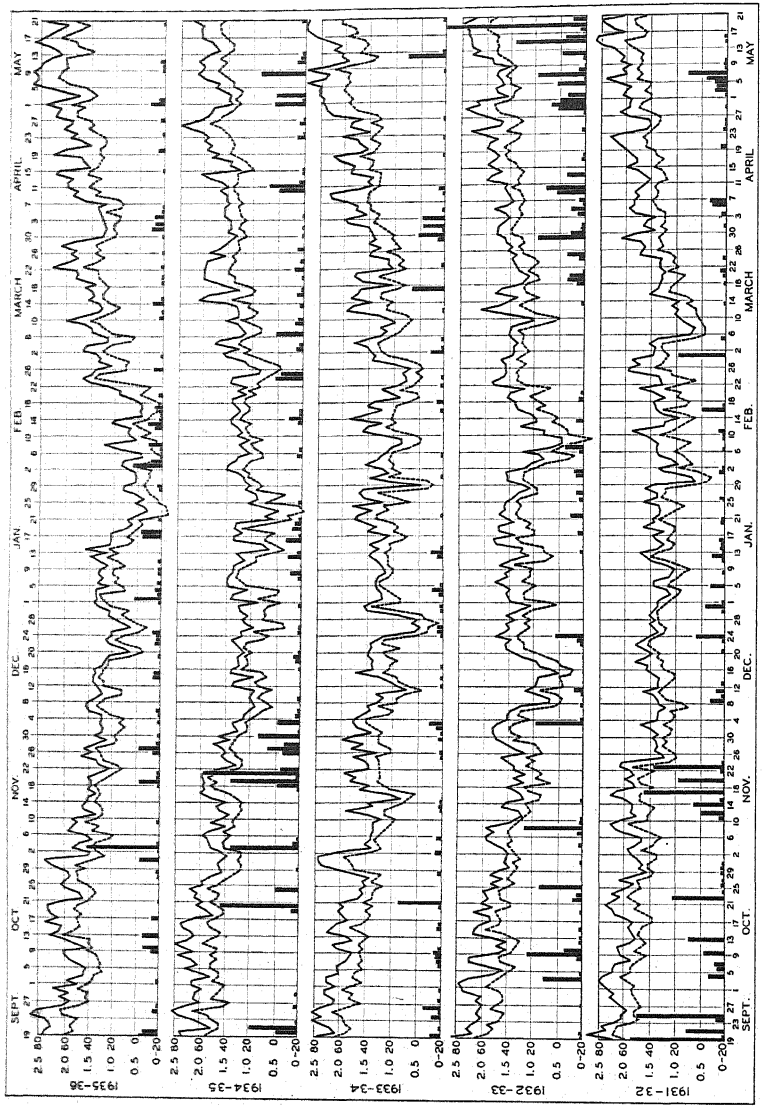


FIGURE 2.—Summary of temperature and rainfall records pertinent to the studies of eradicant spray treatments in relation to apple scab control, Madison, Wis., 1931–36.

In 1931–32, 1933–34, and 1935–36 suppression of the perithecia by eradicant sprays was much easier than in 1932–33 and 1934–35. In 1931–32 the rainfall, though slightly heavier than average, was fairly evenly distributed. In 1932–33 rainfall was slightly above normal in the fall and nearly twice normal in the spring. Rainfall was light in 1933–34, and hot, dry weather in the spring was unfavorable for the fungus. In 1934–35 no rain fell while the treated leaves remained on the trees; but over 10 inches fell during October and November and ample rains occurred in the spring. There were no heavy rains in the fall of 1935, and the following spring was comparatively dry.

EXPERIMENTAL RESULTS

Data on the comparative effectiveness of the experimental materials in suppressing the development of perithecia of *Venturia inaequalis* appear in table 2.

TABLE 2.—*Results of fall spraying experiments for suppression of perithecia of Venturia inaequalis, Madison, Wis., 1931–36*

Formula [1]	Incidence of perithecia in percent [2] of the number [3] found in the untreated leaves of the same varieties in stated years					
	1931, unknown variety	1931, Plumb Cider	1932, Plumb Cider	1933, Plumb Cider	1934, Dudley	1935, Dudley
1. Untreated	100	100	100	100	100	100
2. CS3/4+L1/2+FO1/4	20	16	92	123	55	
3. CS3/4+L1/2+FO1/4					52	
4. CS1+L1+FO1/4			78			
5. CS1+L1/8+FO1/4				106	67	
6. CS1+L1/4+FO1/4				88	49	
7. CS1+L1/2+FO1/4				129	60	
8. CS1+L1/16+FO1/4				123	45	
9. CS1 1/4+L (neut.)+FO1/4			71			
10. CS1 1/2+L1+FO3/8			86			
11. CS1 1/2+L3/8+FO3/8				75	99	
12. CS3/4+L3/4+Cal 1/2+FO1/2			12		5	
13. CS3/4+L1/2+Cal 1/4+FO3/8	2	17	36			
14. CS3/4+L1/2+Cal 3/8+FO3/8	3	21	20		16	
15. CS3/4+L1/2+Cal 1/2+FO3/8		3	19	T		0
16. CS3/4+L1/2+Cal 3/4+FO1/2			20		14	
17. CS3/4+L1/10+Cal 3/8+FO3/8					6	
18. CS3/4+L3/8+Cal 1/4+FO1/4					8	
19. CS3/4+L3/8+Cal 3/8+FO1/4					11	
20. CS3/4+L1/2+Cal 1/8+FO1/4					7	
21. CS3/4+L1/2+Cal 1/4+FO1/4					10	
22. CS3/4+L1/2+Cal 3/8+FO1/4					13	
23. CS1+L1+Cal 1/2+FO3/8			40	5		
24. CS1+L1+Cal 5/8+FO3/8			25			
25. CS1+L1+Cal 3/4+FO3/8			12		3	6
26. CS1+L1+Cal 1+FO3/4			17			
27. CS1+L3/4+Cal 1/2+FO1/2			4			1
28. CS1+L3/4+Cal 5/8+FO1/2			3			
29. CS1+L3/4+Cal 3/4+FO5/8			9		5	
30. CS1+L1/2+Cal 1/4+FO3/8					22	
31. CS1+L1/2+Cal 1/2+FO1/2			4	1	4	
32. CS1+L1/2+Cal 3/4+FO1/2			26			0
33. CS1+L1/8+Cal 1/4+FO1/2				2	30	
34. CS1+L1/8+Cal 3/8+FO1/2				3		
35. CS1+L1/8+Cal 1/2+FO1/2				T		
36. CS1+L1/4+Cal 1/8+FO1/4					24	
37. CS1+L1/4+Cal 1/4+FO3/8				3	26	1
38. CS1+L1/4+Cal 3/8+FO3/8				1	8	1
39. CS1+L1/4+Cal 1/2+FO3/8			1	1	3	0
40. CS1+L1/8+Cal 1/4+FO1/4				4		

[1] CS=copper sulphate; L=lime; FO=fish oil; ZO=zinc oxide. For explanation of other symbols see table 1. The numbers refer to the percent by weight, assuming the mixture to have the same weight as water, with the exception of fish oil, for which they refer to percent by volume. In preparing "neutral" bordeaux, milk of lime was added in successive small amounts until the supernatant liquid, after agitation, no longer gave a positive potassium ferrocyanide test for copper. [2] T=trace.

[3] The average numbers of perithecia per square inch of untreated leaf surface examined were: 1931, unknown variety 553, Plumb Cider 375; 1932, Plumb Cider 575; 1933, Plumb Cider 522; 1934, Dudley 1,188; 1935, Dudley 506. See text for account of methods.

TABLE 2.—*Results of fall spraying experiments for suppression of perithecia of Venturia inaequalis, Madison, Wis., 1931–36*—Continued

Formula	Incidence of perithecia in percent of the number found in the untreated leaves of the same varieties in stated years					
	1931, unknown variety	1931, Plumb Cider	1932, Plumb Cider	1933, Plumb Cider	1934, Dudley	1935, Dudley
41. CS1+L½+Cai ½+FO½				2		
42. CS1+L (neut.)+Cai ½+FO⅜			1			
43. CS1¼+L⅝+Cai ⅜+FO⅜				T		
44. CS1½+L1+Cai ¾+FO¾			23		17	2
45. CS1½+L⅜+Cai ¼+FO½					8	
46. CS1½+L⅜+Cai ⅜+FO½				T		
47. CS1½+L½+Cai ½+FO⅜				1		
48. CS¾+L½+2Cai ½+FO⅜			3			
49. CS¾+L½+2Cai ¾+FO½			17			
50. CS¾+L½+2Cai 1+FO½	1	3	9		5	
51. CS¾+L⅝+2Cai ¼+FO¼					17	
52. CS¾+L⅝+2Cai ⅜+FO¼					15	
53. CS1+L1+2Cai ½+FO⅜			1		22	
54. CS1+L1+2Cai ¾+FO¾			1			
55. CS1+L1+2Cai 1+FO¾			2			
56. CS1+L¼+2Cai ¼+FO⅜					17	
57. CS1+L¼+2Cai ⅜+FO⅜				11		
58. CS1+L¼+2Cai ½+FO⅜				1		
59. CS¾+L½+3Cai ½+FO⅜			5			
60. CS¾+L½+3Cai ¾+FO½			6		14	
61. CS¾+L½+3Cai 1+FO½	4	3	2			
62. CS¾+L⅝+3Cai ⅜+FO¼					7	
63. CS¾+L⅝+3Cai ½+FO¼					13	
64. CS1+L1+3Cai ½+FO⅜			6			
65. CS1+L1+3Cai ¾+FO¾			4	1		
66. CS1+L1+3Cai 1+FO¾			4	6	12	
67. CS1+L½+3Cai 1+FO⅜				0	4	
68. CS1+L½+3Cai ⅝+FO½				1		
69. CS1+L⅝+3Cai ⅝+FO⅜				4		
70. CS1+L¼+3Cai ⅜+FO⅜					24	
71. CS1+L¼+3Cai ½+FO⅜				1	24	
72. CS1+L¼+3Cai ⅝+FO⅜				1		
73. CS1+L¼+3Cai ¾+FO½				9		
74. CS1+L¼+3Cai 1+FO½				T		
75. CS1½+L⅜+3Cai ½+FO½						
76. CS1½+L⅜+3Cai ¾+FO⅜				4		
77. CS¾+L½+Zai ¾+FO½			6			
78. CS¾+L½+Zai 1+FO⅜			18			
79. CS1+L1+Zai ¾+FO⅜					28	5
80. CS1+L1+Zai 1+FO¾			2	T	21	6
81. CS1+L½+Zai 1+FO⅜				31		
82. CS1+L¼+Zai ½+FO⅜					27	
83. CS1+L¼+Zai ¾+FO½				T	10	
84. CS1+L¼+Zai 1+FO½				3		
85. CS1+L⅛+Zai 1+FO½				2		
86. CS1+L⅛+Zai ½+FO⅜				1		
87. CS1+L⅛+Zai 1+FO½				1		
88. CS1¼+L (neut.)+Zai 1+FO½			3			
89. CS1½+L½+Zai ¾+FO⅜					20	
90. CS1½+L½+Zai 1+FO¾				2	16	
91. CS¾+L½+Copai ½+FO⅜	7	12				
92. CS¾+L⅛+Copai ½+FO¼					44	
93. CS¾+L⅛+Copai 1+FO⅜					10	
94. CS1+L¼+Copai ½+FO⅜					34	
95. CS1+L¼+Copai ¾+FO½					19	
96. CS1+L¼+Copai 1+FO½				3	23	1
97. CS1+L⅛+Copai ½+FO⅜					42	
98. CS1+L⅛+Copai ¾+FO⅜					42	
99. CS1+L⅛+Copai 1+FO½				0	20	
100. CS1+L⅛+Copai ½+FO⅜					42	
101. CS1+L⅛+Copai 1+FO½				0	13	2
102. CS1½+L⅜+Copai 1+FO⅜				6	20	
103. CS¾+L⅛+Iai ½+FO¼					34	
104. CS¾+L⅛+Iai ¾+FO⅜					40	
105. CS¾+L⅛+Iai 1+FO⅜					37	
106. CS1+L¼+Iai ½+FO½					54	
107. CS1+L¼+Iai ¾+FO½					26	
108. CS1+L¼+Iai 1+FO¼				1	49	
109. CS1+L⅛+Iai ¾+FO ⅜				1	27	
110. CS1+L⅛+Iai 1+FO½				1	31	

TABLE 2.—*Results of fall spraying experiments for suppression of perithecia of Venturia inaequalis, Madison, Wis., 1931–36*—Continued

Formula	Incidence of perithecia in percent of the number found in the untreated leaves of the same varieties in stated years					
	1931, unknown variety	1931, Plumb Cider	1932, Plumb Cider	1933, Plumb Cider	1934, Dudley	1935, Dudley
111. CS1+L1/16+Iai 1/2+FO3/8				42	42	
112. CS1+L1/16+Iai 1+FO1/2				12	32	
113. CS1 1/2+L3/8+Iai 1+FO5/8						
114. CS3/4+L1/16+PG 1/2+FO3/8			67			
115. CS3/4+L1/16+PG 3/4+FO1/2			41			
116. CS3/4+L1/2+PG 1+FO1/2	T	2	30			
117. CS3/4+L (neut.)+PG 1/2+FO1/4			86			
118. CS1+L1+PG 1+FO3/4			13			
119. CS1 1/2+L1+PG 1/2+FO3/4			63			
120. CS1+L1/4+Lai 1+FO1/2				70		
121. CS1+L1/8+Lai 1+FO1/2				13	32	
122. CS1+L1/16+Lai 1+FO1/2				36		
123. CS1 1/2+L3/16+Lai 1+FO5/8					22	
124. CS1 1/2+L3/8+Lai 1+FO5/8				9		
125. CS1+L1/4+Mai 1/4+FO3/8				6	11	
126. CS1+L1/4+Mai 3/8+FO3/8				13		
127. CS1+L1/4+Mai 1/2+FO 3/8				3		
128. CS1+L1/8+Mai 3/8+FO3/8				1	8	
129. CS3/4+L1/8+2 Caa 1+FO5/8	0	4	19			
130. CS3/4+L1/8+2 Caa 1 1/2+FO5/8			9			
131. CS1+L1+2 Caa 1+FO3/4			40			
132. CS1+L1/2+2 Caa 1+FO5/8			8			
133. CS1+L1/4+2 Caa 1+FO1/2			13			
134. CS1+L (neut.)+2 Caa 1+FO1/2			22			
135. CS1 1/2+L1+2 Caa 1+FO7/8			34			
136. CS3/4+L1/2+3Caa 1+FO1/2		34				
137. CS3/4+L1/4+Cai 1/4+Zai 1/2+FO1/2					5	0
138. CS3/4+L1/4+Cai 1/4+Zai 3/4+FO1/2					12	1
139. CS3/4+L1/2+Cai 1/4+Zai 1+FO5/8			T	T		0
140. CS3/4+L1/8+Cai 1/4+Zai 1/2+FO5/8					11	
141. CS1+L1+Cai 1/4+Zai 1/2+FO5/8					13	
142. CS1+L1+Cai 1/4+Zai 3/4+FO3/4					17	0
143. CS1+L1+Cai 1/4+Zai 1+FO3/4				T	6	T
144. CS1+L1+Cai 1/2+Zai 1/2+FO3/4			1			0
145. CS1+L3/4+Cai 1/4+Zai 1/2+FO5/8						0
146. CS1+L3/4+Cai 1/4+Zai 3/4+FO5/8						5
147. CS1+L3/4+Cai 1/4+Zai 1+FO3/4						1
148. CS1+L3/4+Cai 1/2+Zai 1/2+FO3/4			2			
149. CS1+L3/4+Cai 1/2+Zai 1+FO3/4			4			
150. CS1+L1/2+Cai 1/4+Zai 1/4+FO3/8					16	
151. CS1+L1/2+Cai 1/4+Zai 1/2+FO1/2						6
152. CS1+L1/2+Cai 1/4+Zai 3/4+FO5/8						0
153. CS1+L1/2+Cai 1/4+Zai 1 +FO5/8				0		1
154. CS1+L1/8+Cai 1/8+Zai 1/2+FO1/2					9	
155. CS1+L1/8+Cai 1/8+Zai 3/4+FO1/2					9	
156. CS1+L1/8+Cai 1/4+Zai 1/2+FO1/2					18	
157. CS1+L1/8+Cai 1/4+Zai 3/4+FO1/2				9		
158. CS1+L1/8+Cai 1/4+Zai 1+FO5/8				0		
159. CS1+L1/8+Cai 3/8+Zai 3/4+FO1/2				T		
160. CS1+L1/8+Cai 1/2+Zai 1/2+FO1/2				T		
161. CS1+L1/8+Cai 1/2+Zai 3/4+FO1/2				0		
162. CS1+L3/16+Cai 1/4+Zai 1+FO5/8				0		
163. CS1+L1/4+Cai 1/8+Zai 1/4+FO3/8					33	
164. CS1+L1/4+Cai 1/8+Zai 1/2+FO3/8					20	
165. CS1+L1/4+Cai 1/8+Zai 3/4+FO1/2					18	
166. CS1+L1/4+Cai 1/8+Zai 1+FO1/2				T		
167. CS1+L1/4+Cai 1/4+Zai 1/4+FO3/8					22	
168. CS1+L1/4+Cai 1/4+Zai 1/2+FO1/2				1	8	2
169. CS1+L1/4+Cai 1/4+Zai 3/4+FO1/2				2	6	0
170. CS1+L1/4+Cai 1/4+Zai 1+FO5/8				0		0
171. CS1+L1/4+Cai 3/8+Zai 1/2+FO1/2				5		
172. CS1+L1/4+Cai 3/8+Zai 3/4+FO1/2				0		
173. CS1+L1/4+Cai 1/2+Zai 1/2+FO1/2				1		
174. CS1+L1/8+Cai 1/4+Zai 1/2+FO1/2				T		
175. CS1+L1/8+Cai 1/4+Zai 1+FO1/2				0		
176. CS1+L1/16+Cai 1/4+Zai 1/2+FO3/8				0		
177. CS1+L1/16+Cai 1/4+Zai 1+FO1/2				0		
178. CS1 1/2+L1+Cai 1/4+Zai 1/2+FO3/4						1

TABLE 2.—*Results of fall spraying experiments for suppression of perithecia of Venturia inaequalis, Madison, Wis., 1931–36*—Continued

Formula	Incidence of perithecia in percent of the number found in the untreated leaves of the same varieties in stated years					
	1931, unknown variety	1931, Plumb Cider	1932, Plumb Cider	1933, Plumb Cider	1934, Dudley	1935, Dudley
179. CS1½+L1+Cai ¼+Zai ¾+FO⅞						0
180. CS1½+L1+Cai ¼+Zai 1+FO⅞						0
181. CS1½+L⅜+Cai ¼+Zai ½+FO⅜					29	
182. CS1½+L⅜+Cai ¼+Zai ½+FO⅜					9	
183. CS1½+L⅜+Cai ¼+Zai ¾+FO⅜				T		
184. CS1½+L⅜+Cai ⅜+Zai ½+FO⅜				0		
185. CS1+L1+Cai ½+ZO½+FO¾						T
186. CS¾+L½+Cai ¼+Copai ½+FO½					12	
187. CS1+L1+Cai ½+Copai ½+FO¾						1
188. CS1+L½+Cai ¼+Copai ½+FO½					10	
189. CS1+L½+Cai ¼+Copai ½+FO½					17	
190. CS1+L¼+Cai ⅛+Copai ¼+FO⅜					24	
191. CS1+L¼+Cai ⅛+Copai ½+FO⅜					14	
192. CS1+L¼+Cai ⅛+Copai ¾+FO½					11	
193. CS1+L¼+Cai ⅛+Copai 1+FO½					9	
194. CS1+L¼+Cai ¼+Copai ¼+FO⅜					13	
195. CS1+L¼+Cai ¼+Copai ½+FO½				0	12	
196. CS1+L¼+Cai ¼+Copai ¾+FO½					2	5
197. CS1+L¼+Cai ¼+Copai 1+FO⅝				0		3
198. CS1+L½+Cai ¼+Copai ¾+FO½						1
199. CS1+L½+Cai ¼+Copai 1+FO½						1
200. CS1+L½+Cai ¼+Copai ¾+FO½						T
201. CS1+L½+Cai ¼+Copai 1+FO½						2
202. CS¾+L½+Cai ¼+Iai ¾+FO½					7	
203. CS1+L½+Cai ¼+Iai ¾+FO½					8	
204. CS1+L¼+Cai ⅛+Iai ¼+FO⅜					16	
205. CS1+L¼+Cai ⅛+Iai ½+FO⅜					29	
206. CS1+L¼+Cai ⅛+Iai ¾+FO½					19	
207. CS1+L¼+Cai ⅛+Iai 1+FO½					31	
208. CS1+L¼+Cai ¼+Iai ¼+FO⅜					20	
209. CS1+L¼+Cai ¼+Iai ½+FO½				T	6	
210. CS1+L¼+Cai ¼+Iai ¾+FO½					15	
211. CS1+L¼+Cai ¼+Iai 1+FO⅝				1	10	
212. CS1½+L⅜+Cai ¼+Iai ½+FO⅜					10	
213. CS1+L¼+Cai ⅛+Lai ½+FO⅜					10	
214. CS1+L¼+Cai ⅛+Lai ¾+FO½					10	
215. CS1+L¼+Cai ¼+Lai ¼+FO⅜					22	
216. CS1+L¼+Cai ¼+Lai ½+FO½				0	12	
217. CS1+L¼+Cai ¼+Lai 1+FO½				1		
218. CS¾+L½+Cai ¼+2Cai 1+FO⅝	2	2				
219. CS¾+L½+Cai ¼+3Cai 1+FO⅝	T					
220. CS¾+L½+Cai ¼+2 Caa 1+FO⅝	T					
221. CS1+L1+Cai ¼+2Caa 1+FO¾			16			
222. CS1+L1+Cai ½+2Caa ¼+FO¾			13			
223. CS¾+L½+Cai ¼+3Caa 1+FO⅝	3					
224. CS¾+L½+2 Caa 1+PG ¼+FO⅝			18			
225. CS1+L½+2Caa 1+Zai ½+FO¾			4			
226. CS1+L½+2Caa ½+Zai 1+FO¾			2			
227. CS1+L½+2Caa ¾+Zai ¾+FO¾			1			
228. CS1+L¼+Copai ¼+Iai ½+FO½					29	
229. CS1+L¼+Copai ½+Iai ½+FO½					16	
230. CS¾+L½+Cai ¼+Zai ½+Copai ½+FO⅝						T
231. CS1+L1+Cai ¼+Zai ⅜+Copai ⅜+FO¾						T
232. CS1+L1+Cai ¼+Zai ½+Copai ½+FO¾						T
233. CS1+L¾+Cai ¼+Zai ⅜+Copai ⅜+FO⅝						T
234. CS1+L¾+Cai ¼+Zai ½+Copai ½+FO¾						T
235. CS1+L½+Cai ¼+Zai ⅜+Copai ⅜+FO⅝						2
236. CS1+L½+Cai ¼+Zai ½+Copai ½+FO⅜						9
237. CS1+L¼+Cai ¼+Zai ⅜+Copai ⅜+FO½						3
238. CS1+L¼+Cai ¼+Zai ½+Copai ½+FO⅝						0
239. CS1½+L1+Cai ¼+Zai ½+Copai ½+FO⅞						T
240. CS1+L½+Cai ¼+ZO½+Copai ½+FO⅝						4
241. CS1+L¼+2Cai ⅜+Zai ½+FO½				0		
242. CS1+L¼+3Cai ¼+Zai ¾+FO½				4		
243. CS1+L¼+3Cai ¼+Zai 1+FO⅝				2		
244. CS1+L¼+3Cai ⅜+Zai ½+FO½				10		
245. CS1+L¼+3Cai ¼+Zai ½+FO½				4		
246. CS1+L¼+3Cai ½+Zai 1+FO⅝				10		
247. CS1½+L⅜+3Cai ⅜+Zai ½+FO⅜				11		

Copper-lime mixtures.—The 10 copper-lime formulas used in the copper-lime-arsenic mixtures were tested without the arsenical components. The results were very irregular, varying greatly from year to year. Occasionally a substantial reduction of perithecia was effected. Usually, however, it was less than 50 percent, and in some instances the treatments appeared to be wholly ineffective. None of these preparations was sufficiently effective to have promise for the purposes of this investigation, except for use in mixtures containing some other toxic component.

Copper-lime-monocalcium arsenite mixtures.—Many of the copper-lime-monocalcium arsenite formulas were highly effective in suppressing perithecial development, but host injury was a limiting factor. Many modifications in the concentration and proportions of the ingredients were tried in the hope of finding a formula that would be effective enough and not too injurious. Lowering the amount of lime when the amounts of copper sulphate and the arsenite remained unchanged increased fungicidal effectiveness and host injury. Formulas 15 and 27 are among the best of this group. The development of more promising formulas has recently led to lessening the work on copper-lime-monocalcium arsenite mixtures.

Copper-lime-di- or tricalcium arsenite mixtures.—These mixtures gave results very similar to those of the copper-lime-monocalcium arsenite preparations. The di- and tricalcium arsenites did not appear to be better adapted than monocalcium arsenite for such mixtures.

Copper-lime-zinc arsenite mixtures.—Some copper-lime-zinc arsenite mixtures showed considerable promise. The zinc arsenite, however, had to be used at a concentration of about 1 percent in order to attain a promising degree of effectiveness, and even at this strength these mixtures did not always satisfactorily suppress the development of perithecia. The preparations of this group were distinctly less injurious to the host than the corresponding mixtures in which the calcium arsenites were used. Formula 80 gave good results in 3 of the 4 years in which it was tried. Less attention has recently been given to formulas of this group, since others have seemed to offer more promise.

Copper-lime-copper arsenite mixtures.—The results from copper-lime-copper arsenite mixtures seemed very promising in 1933–34, but were disappointing in the following year. Preparations containing less than 1 percent of copper arsenite seemed to be of comparatively little value. The low-lime mixtures were more efficient than like formulas with higher lime components. Formulas 96, 99, and 101 are among the more promising of this group. These preparations were less injurious to the host than the corresponding mixtures in which calcium or zinc arsenites were used.

Copper-lime-iron arsenite mixtures.—The results from the copper-lime-iron arsenite mixtures were very similar to those from the corresponding preparations in which copper arsenite was used, but in general showed somewhat less effectiveness against the fungus.

Copper-lime-paris-green mixtures.—The copper-lime-paris-green mixtures seemed to be the least promising of the copper-lime-arsenite preparations thus far discussed.

Copper-lime-lead arsenite mixtures.—Copper-lime-lead arsenite mixtures were not sufficiently effective to be of promise.

Copper-lime-magnesium arsenite mixtures.—The results from copper-lime-magnesium arsenite mixtures were similar to those from corresponding preparations in which monocalcium arsenite was used. There was no evidence that magnesium arsenite is better adapted for the purposes of the experiments than the calcium arsenite.

Copper-lime-di- or tri-calcium arsenate mixtures.—The copper-lime-dicalcium arsenate mixtures substantially reduced perithecial development with comparatively little host injury, but they did not appear to be sufficiently effective for the purpose. Substitution of the tri- for the di-calcium arsenate substantially lessened effectiveness against the fungus.

Copper-lime-monocalcium and zinc arsenite mixtures.—This group, in which both a highly soluble and a slightly soluble arsenite are used, contains some of the most promising preparations found in this study. Formula 139 gave almost perfect suppression of the perithecia in each of the 3 years it was used, but host injury occurred to an objectionable degree. Formula 144 gave good control of the fungus in the 2 years it was tried, with less host injury. Formulas 153, 168, 169, and 170 were all highly effective against the fungus, but caused some injury. In a single year's trial formulas 179 and 180 were highly effective and caused only very slight host injury. Further experiments are necessary before conclusions can be reached as to the relative merits of formulas in this group and whether such mixtures can be recommended for use in orchard practice.

Copper-lime-monocalcium arsenite and zinc oxide mixture.—In a single trial, formula 185 suppressed the fungus satisfactorily without objectionable host injury. Further experimentation will be necessary for evaluating this type of preparation.

Copper-lime-monocalcium and copper arsenite mixtures.—Preparations in this group gave results similar to those from the corresponding mixtures in which zinc arsenite was used (formulas 187, 196, and 197), but the suppression of perithecia tended to be less and host injury was about the same.

Copper-lime-monocalcium and other arsenite mixtures.—Iron or lead arsenite seemed less promising than zinc or copper arsenite for addition to copper-lime-monocalcium arsenite mixtures.

Certain miscellaneous mixtures.—While certain miscellaneous mixtures (formulas 219, 220, 226, and 227) caused a substantial reduction in development of perithecia, they seem to be less promising than many other formulas.

Copper-lime-monocalcium, zinc, and copper arsenite mixtures.—In 1 year's tests, certain of these preparations containing three arsenites were highly effective in suppressing the perithecia, and formulas 232, 233, and 234 caused comparatively little injury. However, it seems unlikely that mixtures in this group have as much promise as the copper-lime-monocalcium and zinc arsenite preparations (e. g., formulas 143 and 144).

Copper-lime-di- or tri-calcium and zinc arsenite mixtures.—These preparations, which were tried only in 1933, a year in which suppression of the perithecia was comparatively easy, seem less promising than corresponding mixtures in which monocalcium arsenite was used.

Discussion.—The comparative effectiveness of the several types of formula used has been briefly discussed in the foregoing paragraphs. The results presented show that it is possible, even under very severe conditions of scab development, to reduce the ascigerous stage of the pathogen to a very low level in leaves that are thoroughly sprayed in the fall with suitable eradicant fungicides, ascospore production having been completely suppressed in many individual experiments. These small-scale experiments, however, having been designed chiefly for surveying the comparative effectiveness of many formulas for suppressing the fungus, leave for further investigation numerous questions relating to the possible adaptation of fall spraying as a practical measure for apple-scab control. Some of these are considered below.

VARIATIONS IN TIME OF APPLICATION OF SPRAY

In 1933–34, 1934–35, and 1935–36 a series of experiments was performed to gain data on the relation of time of application to the effectiveness of the sprays against the fungus and their injuriousness to the host. Beginning in late September, applications were made on young Dudley and Northwestern Greening trees (set in the orchard in 1931) at intervals of about 1 week by means of a wheelbarrow sprayer. One or two trees of each variety were given each individual treatment, none being sprayed more than once. Samples of leaves were picked and placed for overwintering on the following dates: 1933, from first and second applications, October 19; from third application, October 24; 1934, from first and second, October 17; third, October 22; 1935, from all applications, leaves picked October 19 and laid on sod October 25. The usual methods of overwintering the leaves and taking data were followed. The results relating to suppression of perithecia appear in table 3.

The variations in time of application employed in these experiments showed no striking or consistent influence on either the effectiveness of the sprays in suppressing the development of perithecia or their injuriousness to the host. In 1933–34 the perithecia were so effectively suppressed by all applications that no substantial differences in effectiveness in relation to the time of application could be observed. There was little injury from any of the treatments. In 1934–35 suppression of the perithecia was unsatisfactory. With the aim of lessening the danger of host injury, the formulas were weakened in a season that proved to be unusually favorable for fungus development. Under the conditions encountered, the latest date of application was the most effective and the intermediate date the least effective. It seems more probable that these differences are due chiefly to relations of time of application to rain periods than that they are attributable to the stage of development of host or parasite. There was little injury from any of the treatments. In 1935–36 the latest application appeared to be slightly more effective than the others. Winter injury in this season was so severe that it interfered with interpretation of results of spray injury. No consistent relation between time of application and host injury was noted.

While these data are too limited to be conclusive, they suggest the desirability of delaying the applications as long as feasible without too much leaf fall.

TABLE 3.—*Effects of variations in the time of application of fall sprays on their efficiency in suppressing perithecia of Venturia inaequalis, Madison Wis., 1933–36*

Year and formula [1]	Incidence of perithecia in leaves sprayed at stated dates in percent [2] of the number [3] found in the untreated leaves					
	Dudley			Northwestern Greening		
	Sept. 21	Sept. 29	Oct. 13	Sept. 21	Sept. 29	Oct. 13
1933–34						
1. Untreated	100	100	100	100	100	100
37. CS1+L¼+Cal¼+FO⅜	1	0	0	1	0	10
38. CS1+L¼+Cal⅜+FO⅜	1	0	1	0	2	3
46. CS1½+L⅜+Cal⅜+FO½	1	1	0	0	0	1
168. CS1+L¼+Cal¼+Zal½+FO½	6	0	0	0	1	1
169. CS1+L¼+Cal¼+Zal¾+FO½	0	0	0	0	0	2
170. CS1+L¼+Cal¼+Zal1+FO⅝	0	0	0	0	0	0
172. CS1+L¼+Cal⅜+Zal¾+FO½	3	0	0	0	0	0
183. CS1½+L⅜+Cal¼+Zal¾+FO⅜	2	0	0	0	0	0
	Sept. 24	Oct. 8	Oct. 22	Sept. 24	Oct. 8	Oct. 22
1934–35						
1. Untreated	100	100	100	100	100	100
95. CS1+L¼+Copal¾+FO½	11	23	13	19	99	17
107. CS1+L¼+Ial¾+FO½	16	45	3	11	47	12
15. CS¾+L½+Cal½+FO⅜	11	28	7	10	93	15
191. CS1+L¼+Cal⅛+Copal½+FO⅜	24	51	2	30	43	4
205. CS1+L¼+Cal⅛+Ial½+FO⅜	15	27	12	6	58	17
164. CS1+L¼+Cal⅛+Zal½+FO⅜	12	42	11	30	39	22
	Sept. 23	Oct. 5	Oct. 10	Sept. 23	Oct. 5	Oct. 10
1935–36						
1. Untreated	100	100	100	100	100	100
25. CS1+L1+Cal¾+FO⅝	12	14	0	1	1	1
143. CS1+L1+Cal¼+Zal1+FO¾	2	2	1	1	2	1
236. CS1+L½+Cal¼+Zal½+Copal½+FO⅝	0	3	0	1	0	T
197. CS1+L¼+Cal¼+Copal1+FO⅝	4	5	T	25	T	2

[1] For explanation of symbols used see footnote 1, table 2.
[2] T=Trace.
[3] The average numbers of perithecia per square inch of untreated leaf surface examined were: 1933, Dudley 1003, Northwestern Greening 370; 1934, Dudley 493, Northwestern Greening 214; 1935, Dudley 506, Northwestern Greening 364.

SPRAY INJURY TESTS UNDER ORCHARD CONDITIONS

Extensive data on spray injury were taken in connection with the small-scale experiments on suppression of perithecia. However, the conditions of most of these experiments were so unsatisfactory for injury studies that the results relating thereto will not be given in detail. While more extensive studies on injury must await further narrowing of the problem, some experiments have been initiated, as reported below.

Thirteen different copper-lime-arsenic formulas (nos. 15, 24, 39, 80, 95, 107, 143, 164, 169, 170, 191, 197, and 205 of table 2) were applied during the first half of October by power sprayers to orchard trees of eight apple varieties (Wealthy, McIntosh, McMahon, Northwestern Greening, Fameuse, Lowland Raspberry, Dudley, and Yellow Transparent) in 40 treatments at Sturgeon Bay, Gays Mills, or Madison during the period from 1932–33 to 1935–36, inclusive. One or several trees were used per treatment. Examination was made for leaf injury

about 1 or 2 weeks after the sprays were applied and for bud injury the following spring. No drastic leaf injury was observed, except in one case when showery weather prevented prompt drying of the sprays. Observation of the sprayed and unsprayed trees in the spring revealed no evidence of significant spray injury to the buds. Counts relating to bud injury were made only on Wealthy at Madison. These revealed no significant difference between the sprayed and unsprayed trees, except possibly for formula 39 in 1935–36. In this case the untreated trees showed 2 percent and the sprayed 10 percent of dead terminal buds. Winter injury affected both sprayed and unsprayed trees this year.

These results, though encouraging, are not interpreted as establishing the safety of these formulas for general use as fall sprays for apples.

Effects of Eradicant Spraying on the Epidemiology of Scab

The work thus far reported seems to indicate substantial possibilities for the development of a fall spray treatment capable of reducing the ascosporic inoculum to the neighborhood of 1 percent, or less, of that from similar untreated leaves. The effects of such reductions on the epidemiology and control of the disease remain to be considered. Only a small beginning has been made in experimentation in this important field. Studies of the effects of certain fall spray treatments on the development of the disease in the following year were begun at Madison in 1934. A brief report of the progress of this work follows:

Orchard no. 1 of figure 3, set in 1930, received the fall sprays. Orchard no. 2, set in 1931, was untreated. Both orchards were vigorous. Neither received summer sprays. Cultivation of both was postponed until ascospore discharge was over.

Experiment of 1934–35

On October 9, 1934, all of orchard no. 1 was sprayed by means of a power machine, formulas 191, 164, and 205 of table 2 being used on different subplots.

The environment in fall, winter, and spring was favorable for overwintering of the fungus. Examinations in the spring showed that the sprayed orchard was very nearly free from perithecia of the scab fungus, whereas a fair amount of ascosporic inoculum had developed in the unsprayed. Ascospores were mature by the time the leaf buds opened, and several days of rain and low temperature afforded favorable conditions for early infection (fig. 4). Scab lesions were first observed on May 29, when a wave of disease began to appear. Rains in June and July permitted further infection periods, and the season was generally favorable for scab development.

Comparative data on scab development in the two orchards appear in tables 4 and 5. The results shown in table 4 indicate that through the month of June reduction of scab infection in the sprayed orchard was not less than 90 percent. The sprayed Wealthy block showed 92 percent scab-free fruit on June 24, as compared with 48 percent from the unsprayed (table 5). At harvest 89 percent of the fruit from the sprayed block was free of scab, while the unsprayed block had insufficient fruit for counts at that time.

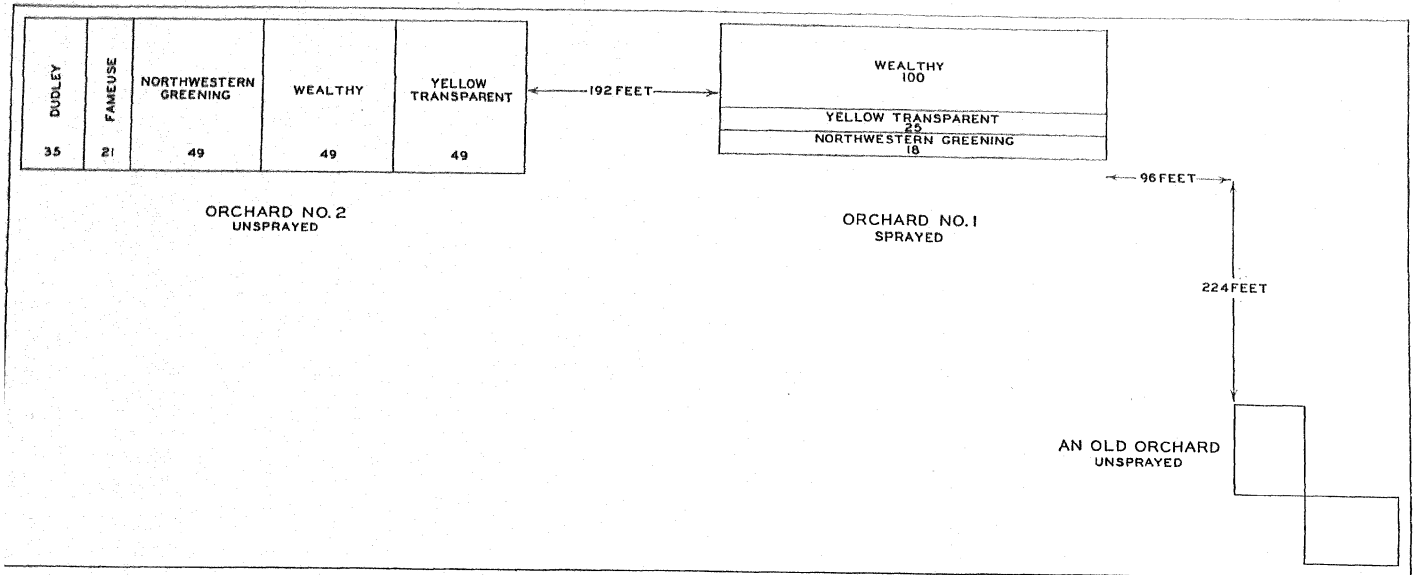


FIGURE 3.—Map of experimental orchards used in studies of the effects of eradicant spray treatments on the development of apple scab Madison, Wis., 1934-36. The figures in the several plots record the number of trees.

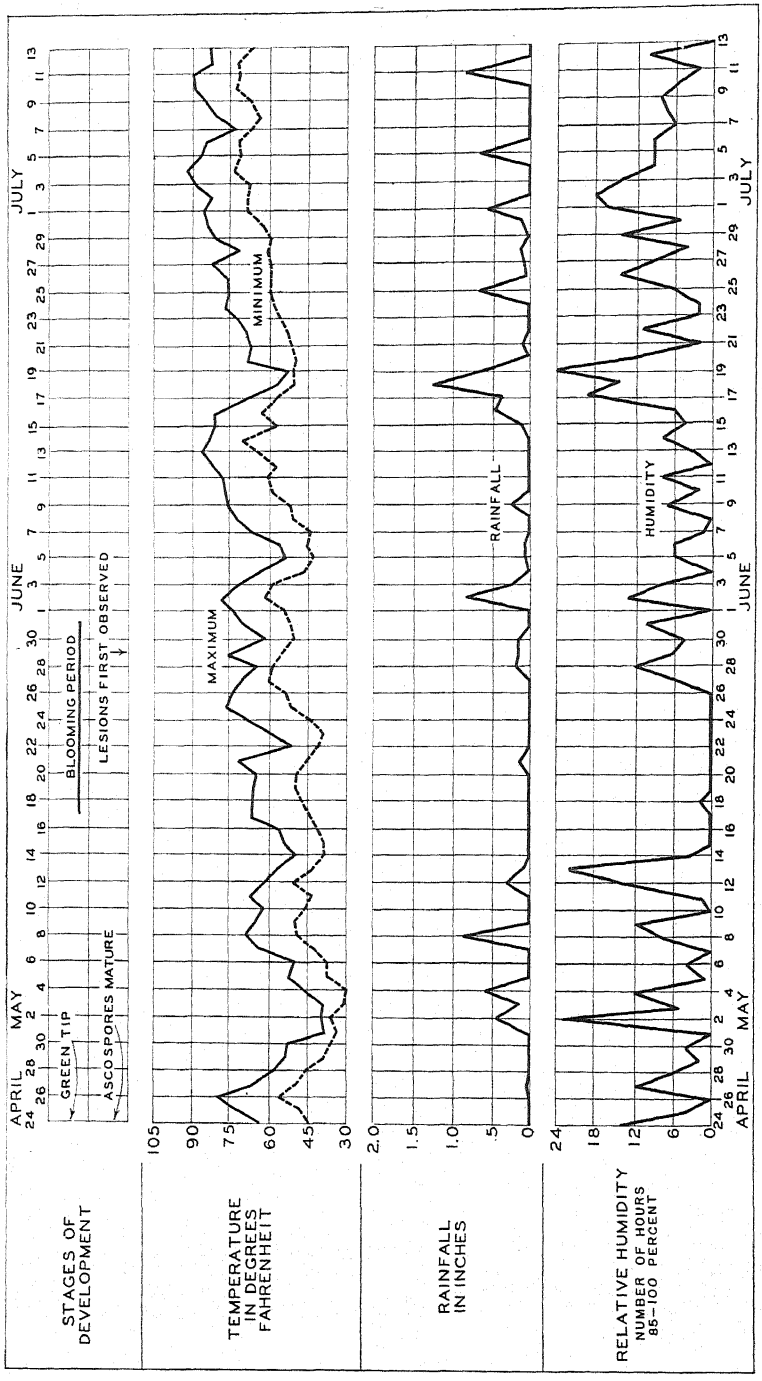


FIGURE 4.—Summary of certain records relating to the development of apple scab on Wealthy, Madison, Wis., 1935.

TABLE 4.—*Effects of fall spraying with eradicant fungicides on the development of apple scab in the following season, Madison, Wis., 1934–35 and 1935–36*

Year, variety, and date	Counts made on—	Orchard no. 1, sprayed [1]		Orchard no. 2, unsprayed		Decrease in disease in orchard 1 as compared with orchard 2
		Total units examined	Average lesions per unit	Total units examined	Average lesions per unit	
1934–35						
Wealthy:		*Number*	*Number*	*Number*	*Number*	*Percent*
May 31	Fruit spurs (leaf lesions)	509	0.078	250	1.708	96
June 24	Fruit	270	.42	113	4.50	91
June 29	Leaves of terminal shoots	283	.52	274	4.8	89
Northwestern Greening, June 29	do	293	.72	282	18.0	96
1935–36						
Wealthy, June 2	do	2,575	.007	1,839	.648	99
Northwestern Greening, June 4	do	1,219	.047	1,230	.832	94
Wealthy, June 6	do	3,042	.48	2,387	3.67	87
Northwestern Greening, July 9	do	1,450	.71	1,464	4.88	85
Wealthy:						
July 10	Fruit	200	.075	200	.57	87
Sept. 2	do	1,334	1.93	718	7.79	75

[1] Orchard no. 1 sprayed in 3 subplots on Oct. 9, 1934, with formulas nos. 164, 191, and 205 of table 2; on Oct. 9, 1935, with formulas nos. 39, 143, and 152 (modified to contain zinc arsenite at ½ of 1 percent). No summer spray was applied to either orchard.

TABLE 5.—*Effects of fall spraying with eradicant fungicides on the development of apple scab in the following season, Madison, Wis., 1934–35 and 1935–36*

Year, variety, and date	Counts made on—	Orchard no. 1, sprayed [1]		Orchard no. 2, unsprayed	
		Total units examined	Units free of scab	Total units examined	Units free of scab
1934–35					
Wealthy:		*Number*	*Percent*	*Number*	*Percent*
June 24	Fruit	270	92	113	48
June 29	Terminal shoots (leaf lesions)	20	45	20	0
Northwestern Greening, June 29	do	20	50	20	0
Wealthy, Sept. 4	Fruit	320	89	(2)	.....
1935–36					
Wealthy, June 2	Terminal shoots (leaf lesions)	207	95	156	3
Northwestern Greening, June 4	do	100	66	100	1
Wealthy:					
June 8	Fruit	100	100	100	97
July 6	Terminal shoots (leaf lesions)	207	17	156	0
Northwestern Greening, July 9	do	100	11	100	0
Wealthy:					
July 10	Fruit	200	97	200	72
Sept. 2	do	1,334	45	718	1

[1] Orchard no. 1 sprayed in 3 subplots on Oct. 9, 1934, with formulas nos. 164, 191, and 205 of table 2; on Oct. 9, 1935, with formulas nos. 39, 143, and 152 (modified to contain zinc arsenite at ½ of 1 percent. No summer spray was applied to either orchard.

[2] No fruit available for counts.

EXPERIMENTS OF 1935–36

On October 9 all of orchard no. 1 was sprayed, formulas 39, 143, and 152 (modified to contain zinc arsenite, one-half of 1 percent) of table 2 being used on different subplots. Orchard no. 2 was un-

sprayed, except the Yellow Transparent block, which was given the same treatments as orchard no. 1 in order to increase the distance between that orchard and the source of ascosporic inoculum in the unsprayed orchard.

Conditions were generally favorable for production of perithecia of the fungus, although dry weather in the spring somewhat delayed ascospore discharge. Counts made by the usual method showed that in the plots treated, respectively, with formulas 39, 143, and 152, Wealthy leaves averaged 3, 4, and 9 perithecia per square inch of leaf surface examined. In the unsprayed orchard the leaves averaged 255 perithecia per square inch of leaf surface examined for Wealthy, 364 for Northwestern Greening. It appears, therefore, that production of perithecia in the sprayed orchard was reduced by approximately 98 percent.

Ascospores were ready for discharge by May 3. The first infection period occurred May 9–11 (fig. 5), and scab lesions were first observed May 29. The rains of late May and June permitted moderate infection. The development of the disease was sharply checked by hot, dry weather in July. Frequent periods of rain in August favored infection.

Beginning June 6, the seasonal development of the disease was followed on 20 tagged terminal shoots on as many trees in each orchard. The results are shown in figure 5.

Further comparative data on scab development in the sprayed and unsprayed orchards appear in tables 4 and 5. The results shown in table 4 indicate that through June and early July reduction of scab in the sprayed orchard was not less than 85 percent. Wealthy fruit of the sprayed orchard at harvest time showed a reduction of 75 percent. On June 2, 95 percent of the Wealthy terminal shoots in the sprayed orchard were free from scab lesions, against 3 percent in the unsprayed (table 5). On July 6, 17 percent were scab-free in the sprayed orchard, none in the unsprayed. On June 8 all the Wealthy fruit in the sprayed orchard was scab-free, against 97 percent in the unsprayed. At harvest 45 percent of the fruit of the sprayed orchard remained free from scab, against 1 percent in the unsprayed.

When this experiment was planned it was realized that the available orchards were too near together to preclude the probability of passage of a substantial ascosporic inoculum from the unsprayed to the sprayed. In view of this fact, the differences in scab development are the more striking.

In order to gain evidence concerning the possible influence of inoculum from the unsprayed orchard on disease development in the sprayed, the incidence of scab in the latter orchard was studied in relation to distance from the former (fig. 6). On July 6 data were taken on 50 Wealthy terminal shoots in each of the distance classes shown in figure 6. The results show that the incidence of infection diminished substantially with increased distance, an average of 12 lesions per terminal shoot occurring in the least distant class and 3 in the most distant. Conversely, the percent of terminal shoots free of scab increased with distance from 6 in the least distant class to 34 in the most distant.

Exact data on the distance to which ascospores of *Venturia inaequalis* may be carried in the air and the relations of distance from the source of the ascosporic inoculum to its concentration and to the

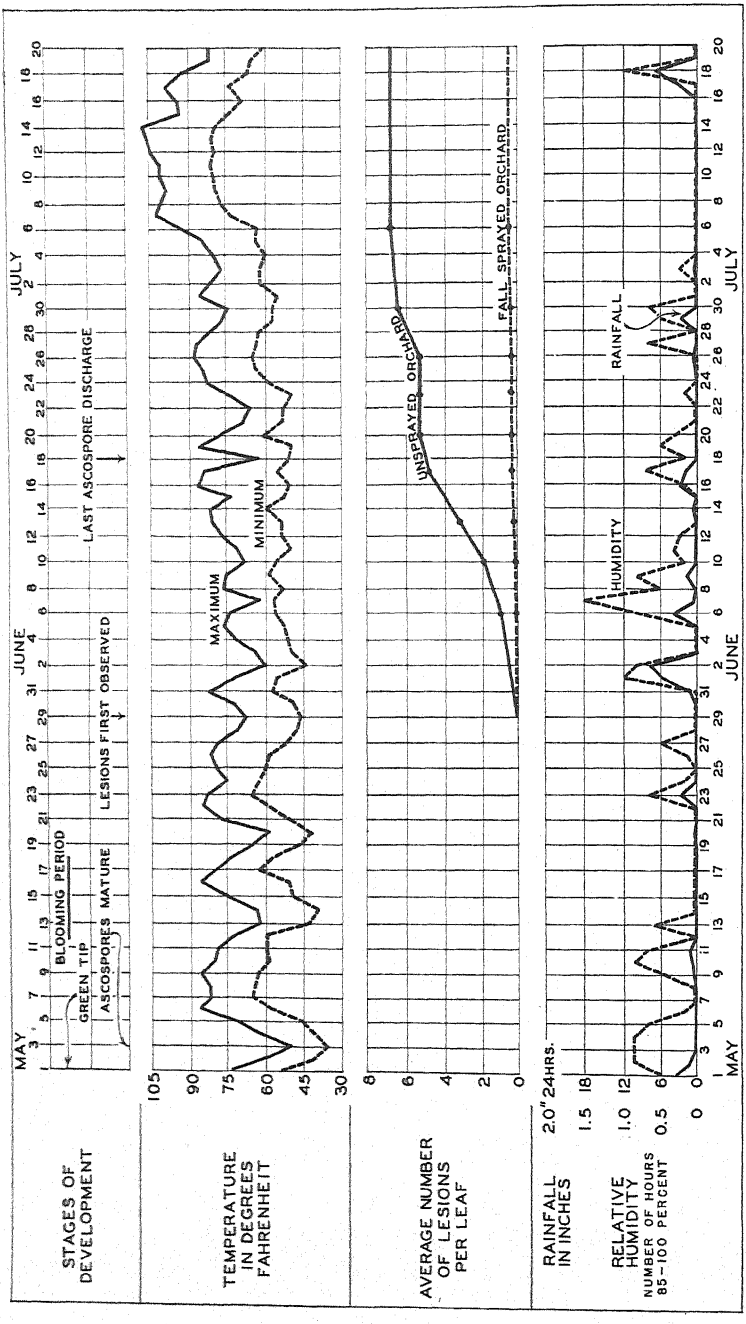


FIGURE 5.—Summary of certain records relating to the development of apple scab on Wealthy, Madison, Wis., 1936.

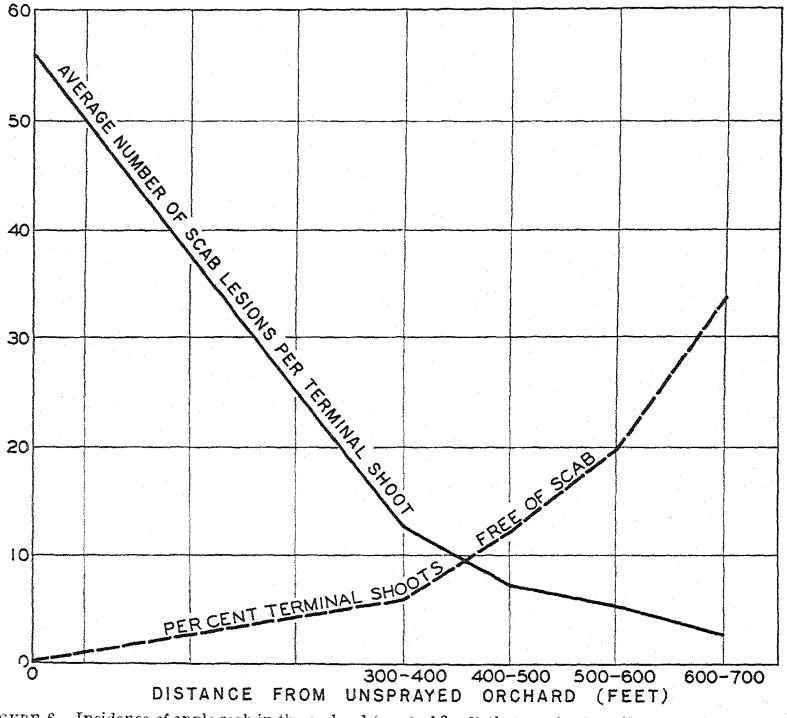


FIGURE 6.—Incidence of apple scab in the orchard (no. 1 of fig. 3) that received a fall treatment in relation to distance from the unsprayed orchard (no. 2), which contained an abundant ascosporic inoculum, Madison, Wis., 1936.

incidence of infection caused by it are meager. Undoubtedly the spores may be carried for great distances in the air (*23*). However, the limited evidence from the experiment that has just been reported, together with theoretical considerations and orchard observations, suggests that the concentration of this air-borne inoculum and the incidence of disease that it may cause diminish rapidly with the distance it is carried.

DISCUSSION

In the consideration of the results of the foregoing experiment, attention is invited to the fact that these fall treatments were not designed or expected to obviate the necessity of summer spraying. The summer sprays were omitted in order that effects of fall spraying might be studied more advantageously.

It should be observed that the seasonal conditions encountered in the course of this experiment did not favor heavy infection during the critical preblossom and blossom periods. Much more work under a wide range of conditions will be necessary to arrive at an adequate understanding of the relations of the quantitative level of the ascosporic inoculum to the epidemiology and control of the disease. The limited experimental data thus far obtained are in full accord with the evidence of earlier studies (*16*) in indicating that these relations are highly significant.

Seasonal conditions at Gays Mills,[6] Wis., in 1934 and 1935 afforded an opportunity for certain observations on the relations of a greatly reduced ascosporic inoculum to the seasonal development and control of scab. The extreme conditions of heat and drought in 1934, in conjunction with efficient summer spraying, so reduced the incidence of scab that perithecia of the pathogen were hard to find in certain large blocks of trees in the spring of 1935. The fungus, however, overwintered at a somewhat higher survival level in some situations where the spraying had been less thorough or the local environment more favorable, as in certain ravines. In 1935 conditions of moisture and temperature were very favorable for scab development, until late in the season. With a normal ascosporic inoculum a very severe and difficultly controllable epidemic of scab would have been expected. Actually, the disease development was moderate, and control was comparatively easy, especially in certain blocks where the ascosporic inoculum was known to have been very sparse. In one Dudley block in which the fungus was reduced to a very low survival level, only 49 percent of the unsprayed fruit showed scab at harvest; in a block of the same variety in which scab had been somewhat more prevalent the year before, 98 percent was scabbed. Unsprayed McIntosh in an orchard in which the fungus had overwintered at a very low survival level had only 88 percent of the fruit scabbed and 30 percent rendered unmarketable because of the disease. In this orchard a lime-sulphur program that began with the open-cluster spray reduced the percentage of scabby fruit to 2, and a like program beginning with the calyx application reduced it to 17, of which three-fourths could go into the U. S. No. 1 grade. This experience accords well with other data and with theoretical considerations in suggesting that a sufficient reduction in the ascosporic inoculum may be a potent factor in delaying and diminishing scab development and lessening the difficulty of its control by protectant sprays.

Chemical Treatment of Leaves on the Ground

In situations in which overwintering of *Venturia inaequalis* depends wholly or dominantly on production of the ascigerous stage, the period after leaf fall and before ascospore discharge is, obviously, a potentially vulnerable one. The fungus is then prostrate on the floor of the orchard. If it were as conspicuously visible as Canada thistle (*Cirsium arvense* (L.) Scop.), for example, would we continue to permit this dangerous and expensive pest to breed year after year in our orchards, essentially unmolested? Considerable progress has been made in the development of eradicant chemical treatments for use against weeds. Possibilities of adapting similar methods of chemical eradication to the control of apple scab and some other fungus diseases seem to deserve consideration.

Experiments with liquid applications of certain fertilizers that are known to have toxic properties and with certain weed killers have been initiated. In preliminary experiments in the spring of 1936, saturation of apple leaves as they lay on the ground by spraying with ammonium sulphate dissolved in water at the rate of 1 pound to the gallon killed the ascospores that were mature and prevented the maturation of others. Further results from this line of work are not yet available.

[6] Dr. J. A. Pinckard collaborated in this part of the investigation.

Present Status of the Work on Apple Scab

These studies on apple scab may be considered first in relation to the control of this disease, and second with reference to the broader question of the potentialities of eradicant fungicides for increased use against other plant diseases. The first of these aspects of the work is discussed in the following paragraphs, the second in a later section of this paper.

The problem relating directly to apple scab control has three major phases: (1) The development of means for sharply limiting the ascosporic inoculum; (2) studies of the effects of such limitation of the inoculum on the epidemiology and control of the disease; and (3) large-scale experiments to determine whether the procedures developed can be adapted to comprise a part of an improved program of scab control. Attention has thus far been devoted to the first and second phases. The results seem to have demonstrated that, under conditions such as were encountered in these experiments, (1) suitable applications of eradicant fungicides are capable of drastically reducing the ascosporic inoculum, and (2) such reduction greatly retards and ameliorates the development of the disease, especially in the critical period of primary and early secondary infection.

It is recognized that many obstacles must be overcome if fall spraying is to find a place in the apple scab control program. The most important appears to be the problem of host injury. Many of the formulas employed appear to be on the threshold of satisfactory fungicidal efficiency, without too much injury to the host. However, extensive tests, with due consideration to the effects of different environments and to varietal responses, will be necessary to establish the safety of such materials for use in orchard practice.

Other obstacles, such as early leaf fall under some conditions, difficulty of thorough coverage of the foliage of large trees, the inconvenience of spraying after harvest, etc., must also be considered. These difficulties vary much in importance with conditions in different regions. They do not seem to be insurmountable under ordinary Wisconsin conditions. It is scarcely to be expected that any control program will be adapted to all regions in which scab occurs.

Such eradicant chemical procedures as are here discussed can be established as a part of a sound program for apple scab control only if and when they justify themselves on the basis of extensive trial under orchard conditions. Much work remains to be done in developing methods and studying their effects before large-scale tests are warranted. Unless or until these methods stand the test of such trials, growers are advised to refrain from trying them.

The studies on apple scab are being continued.

EXPLORATORY STUDIES ON OTHER DISEASES

Though this investigation has been centered about apple scab, as an important disease that afforded opportunity and material for the work, its larger purpose has been to inquire into possibilities for wider application of the principle of eradication to plant disease control through the use of chemical agents. After it became evident that suitable applications of copper-lime-arsenic mixtures were capable of preventing the development of ascocarps of *Venturia inaequalis*, certain exploratory experiments were initiated to gain evidence

concerning their potentialities for limiting the development or the dispersal of an effective primary inoculum of certain other pathogenic fungi. A brief report of this work, which was necessarily limited in extent, follows. It seems unnecessary to burden this paper with a review, for each of the diseases dealt with, of the previous efforts in this direction, in which other materials, chiefly those adapted primarily for summer spraying, were employed.

BROWN ROT OF PLUM

On April 29, 1933, formulas CS1½+L¼+Cai¾+FO⅝ and CS1½+L⅜+Cai1+FO¾ (for explanation of symbols, see footnote 1, table 2) were applied to branches of *Prunus domestica* L. bearing persistent mummified fruits that had been attacked by the brown rot fungus, *Sclerotinia fructicola* (Wint.) Rehm, in the preceding year. The blossom buds had expanded but not separated in the clusters. Sprayed and unsprayed mummies, collected on May 5, were examined by a standardized procedure for occurrence of conidia. None were found on the sprayed mummies, whereas they occurred in abundance on the unsprayed. In a similar experiment in the following year formulas 10 and 12 of table 6 gave like results. No spray injury was observed to follow these treatments.

APPLE BLOTCH [7]

In 1934 and 1935 individual branches of three large Northwestern Greening apple trees bearing abundant twig lesions caused by the blotch fungus, *Phyllosticta solitaria* E. and E., were treated at Urbana, Ill., by means of hand sprayers.

In 1934 the trees were at the green-tip stage when the sprays were applied. Light showers occurred the night after the applications, followed by dry weather for several weeks. On June 2 and 14 twigs were collected at random from the treated and untreated branches and sent to Madison, where they were held for 5 days in a moist chamber at 20° C. to give the pycnidia further opportunity to mature. The percentage of lesions that bore any sporulating pycnidia was then determined by microscopic examination. Results from old lesions and those from new lesions and advancing margins of old ones were taken separately, the current year's advancing margins of an old lesion being counted as a new lesion.

In 1935 the experiment was repeated, with modifications. There were two dates of application, and the twigs were collected for examination on May 2.

A summary of the treatments and results for both years' experiments appears in table 6.

In 1934 the sprays were applied too late for maximal effectiveness. Nevertheless, formulas 1 and 2 seemed to prevent sporulation on about three-fourths of the old lesions (fig. 7, A, B). There was comparatively little reduction of sporulation on new lesions. Lime-sulphur and bordeaux treatments were comparatively ineffective. Formula 1 caused perceptible host injury; the others did not.

[7] The writers are indebted to Dr. H. W. Anderson of the Illinois Agricultural Experiment Station for his helpful cooperation in this part of the work and for permission to report the results, and to Fred Heaton of New Burnside, Ill., for making his orchard and spraying facilities available for the work of 1936.

TABLE 6.—*The effectiveness of certain spray treatments in suppressing sporulation of Phyllosticta solitaria in twig lesions of Northwestern Greening apple, Urbana, Ill., 1934 and 1935*

Date of application	Formula [1]	Total twigs observed	Old [2] lesions			New [2] lesions		
			Total	Bearing sporulating pycnidia		Total	Bearing sporulating pycnidia	
		Number	*Number*	*Number*	*Percent*	*Number*	*Number*	*Percent*
1934	0. Untreated	10	15	15	100	16	16	100
Apr. 11	1. CS1½+L⅜+Cal 1+FO⅜	11	19	5	26	15	13	87
Do	2. CS1½+L⅜+Cai¾+Zai¾+FO¾	9	17	4	24	17	15	88
Do	3. CS1½+L¾+Cai¾+Zai¾+FO⅞	10	11	4	36	25	20	80
Do	4. CS1+L¼+Cai ½+Zai ½+FO½	5	10	9	90	10	10	100
Do	5. CS1+L⅛+Cai ½+Zai ½+FO½	7	14	10	72	24	24	100
Do	6. CS1+L⅛+Zai 1+FO½	9	22	15	68	13	13	100
Do	7. CS1½+L⅜+FO½	10	19	14	74	19	19	100
Do	8. Lime-sulphur 1–7	9	22	21	96	13	13	100
1935	9. Untreated	12	49	49	100	17	12	71
Feb. 14	10. CS1½+L⅜+Cai¾+Zai¾+FO¾	6	42	0	0	--------	--------	--------
Apr. 8	do	15	45	12	27	12	4	33
Feb. 14	11. CS1½+L⅜+Cai ¾+Copai ¾+FO¾	7	68	2	3	3	0	0
Apr. 8	do	7	30	5	17	2	1	50
Feb. 14	12. CS1½+L¾+Cai¾+Zai¾+FO¾	4	31	4	13	4	0	0
Apr. 8	do	10	30	26	87	4	0	0

[1] For explanation of symbols, see footnote 1, table 2.
[2] See text.

In 1935 the treatments applied February 14 were decidedly more effective than those of April 8. No sporulating pycnidia were found on old lesions that received the early application of treatment 10, and treatments 11 and 12 were nearly as effective, with the same timing.

Under the conditions of the experiment, few new lesions developed on the experimental branches. The data on these are too meager to offer more than a suggestion that there was some measure of limitation of sporulation on young lesions, especially by the earlier treatments.

In 1936 an experiment in which the sprays were applied by a power machine was performed at New Burnside, Ill. Applications were made March 4 on 10- to 15-year-old Duchess trees that bore abundant blotch lesions, especially on the fruit spurs. Each program of treatments was used on two or more trees. The experiment was planned primarily to gain evidence on the effects of the several programs on the incidence of blotch on fruit, but, owing to cold injury, practically no fruit was set. Furthermore, on account of the very dry season, there was no significant amount of new infection, even on unsprayed trees. Samples of twigs collected May 27 were examined microscopically on May 28 with reference to the percentage of pycnidia that bore spores. The results appear in table 7.

The examinations showed that 42 and 65 percent, respectively, of the pycnidia in the old lesions from the two plots (1, 2) that received no dormant spray contained spores; none in the old lesions of plots (3–8) that received the dormant treatment.

Where the dormant treatment was omitted (plots 1, 2), 100 percent of the pycnidia in the new lesions bore spores. In three of the four samples (plots 3, 5, 7, 8) that received both the dormant spray and the summer applications of bordeaux, only about half of the pycnidia

in the new lesions sporulated. In the samples that received the dormant treatment without the summer applications of bordeaux (plots 4, 6) the percentage of sporulating pycnidia was slightly higher.

TABLE 7.—*The effectiveness of certain spray treatments in suppressing sporulation of Phyllosticta solitaria in twig lesions of Duchess apple, New Burnside, Ill., 1936*

Plot no.	Formulas [1] applied Mar. 4, 1936	Summer treatment [2]	Type [3] and number of lesions observed	Total pycnidia observed	Pycnidia with spores	
				Number	*Number*	*Percent*
1	Untreated	Lead arsenate only	Old 5	57	24	42
			New 11	33	33	100
2	do	Bordeaux plus lead arsenate	Old 3	17	10	65
			New 7	24	24	100
3	CS1½+L⅜+Cai ¾+Zai ¾+FO¾	do	Old 5	16	0	0
			New 19	55	27	49
4	do	Lead arsenate only	Old 5	20	0	0
			New 19	54	38	70
5	CS1+L⅜+Cai ¾+Zai ¾+FO¾	Bordeaux plus lead arsenate	Old			
			New 8	30	14	47
6	do	Lead arsenate only	Old 3	15	0	0
			New 7	27	16	59
7	CS1½+L⅜+Cai 1+Zai ½+FO¾	Bordeaux plus lead arsenate	Old 5	19	0	0
			New 9	23	23	100
8	CS1+L1+Cai ½+Zai 1+FO¾	do	Old 3	13	0	0
			New 16	41	20	49

[1] For explanation of symbols, see footnote 1, table 2.
[2] All plots were sprayed with flotation sulphur, 16–100, just before the blossoms opened, at petal fall, and 1 week later. Lead arsenate and hydrated lime, each 3–100, were added at petal fall. The bordeaux plots received applications of bordeaux, 4–6–100, plus lead arsenate, 4–100, at 2 and 3 weeks after petal fall. The other plots were treated at the same dates with lead arsenate and hydrated lime, each 4–100.
[3] See text.

None of the dormant spray treatments caused any perceptible host injury.

While the available data are very meager, they indicate that suitable applications of copper-lime-arsenic mixtures are capable of preventing sporulation in a high percentage of the pycnidia of the blotch pathogen, especially those borne in the old lesions. Whether these or similar treatments can be sufficiently perfected to become a practical measure for blotch control lies beyond the scope of the present investigation.

PEACH SCAB

On February 16, 1933, formulas 25, 30, and 31 of table 2 and bordeaux mixture, 4–2–50, were each applied at Madison by means of hand sprayers to twigs of *Amygdalus persica* L. bearing lesions caused by *Cladosporium carpophilum* Thüm. on the growth of the preceding year. On March 4 the twigs were collected and placed in a moist chamber at 22°–24° C. for 3 days to favor sporulation. They were then examined microscopically by a standardized procedure for incidence of conidia of the scab pathogen. The untreated lesions bore abundant conidia. The following percentage reductions in the number of spores borne on the treated lesions, based on the results from the untreated, were indicated: Formula 25, 100; formula 31, 99.2; formula 30, 93; bordeaux, 4–2–50, 60. Twigs sprayed with the copper-lime-arsenic mixtures showed some injury after growth was resumed, chiefly manifested as a roughening of the bark. The peach plant is well known to be very sensitive to copper injury. Lack of scab-infected peach trees precluded further experiments at Madison.

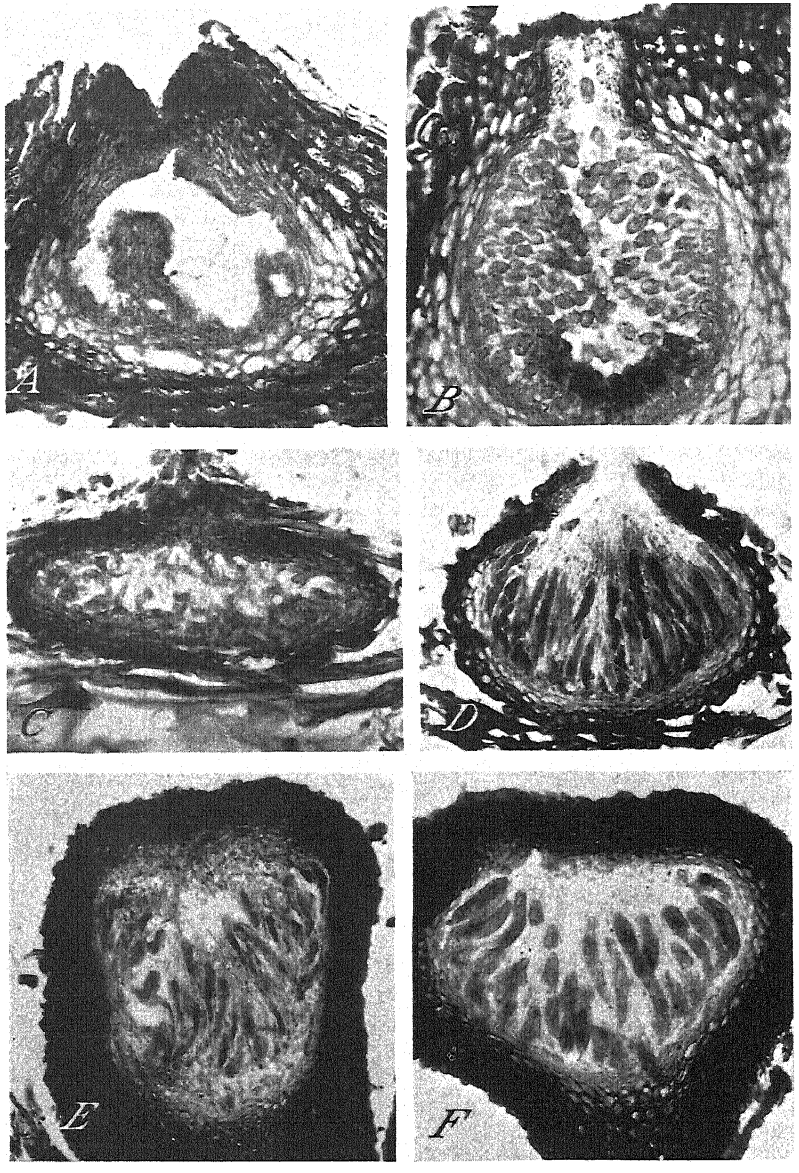


FIGURE 7.—Sections of fungal fruiting structures from fixed material, showing effects of eradicant spray treatments: *A*, Disorganized pycnidium of *Phyllosticta solitaria* 2 months after treatment with formula 2 of table 6; *B*, as *A* but untreated; *C*, disorganized ascocarp of *Mycosphaerella rubina* 17 days after treatment with a copper-lime-arsenic mixture; *D*, as *C* but untreated; *E*, disorganized ascocarp of *Dibotryon morbosum* 21 days after treatment with formula 12 of table 8; *F*, as *E* but untreated. All approximately × 250.

On March 15, 1935, three copper-lime-arsenic mixtures were applied to peach trees at Lexington, Ky.[8], by means of a power sprayer. The flower buds had already begun to show pink. Examination of twigs collected May 17 showed that the sprayed lesions bore about one-third to one-half as many conidia as the unsprayed. It seems probable that the lateness of application and the use of weaker mixtures may account in large measure for the difference between the Kentucky results and those obtained at Madison.

These data, though fragmentary and inconclusive, suggest possibilities of substantial reduction of sporulation of the peach scab fungus in twig lesions by means of suitable dormant spraying, and indicate that the copper-lime-arsenic sprays were far more effective than the bordeaux.

SPUR BLIGHT OF RASPBERRY

At Madison on May 15, 1933, several copper-lime-arsenic mixtures were applied by hand sprayers to canes of red raspberry (*Rubus strigosus* Michx.) affected by spur blight, caused by *Mycosphaerella rubina* (Pk.) Jacz. Microscopic examination of samples collected May 29 showed that perithecia on the sprayed canes were dead, whereas those on the unsprayed were in good condition. When fragments of moist bark bearing numerous perithecia were placed over clear agar in Petri dishes, there was abundant discharge of ascospores from the unsprayed material, none from the sprayed. Histological sections of sprayed and unsprayed perithecia collected June 1 are illustrated in figure 7, *C*, *D*.

PEAR SCAB

In a single small-scale experiment at Madison in 1933–34, pear (*Pyrus communis* L.) branches of an unknown variety bearing scabby leaves were treated in the fall with certain copper-lime-arsenic mixtures to test the effectiveness of the latter in suppressing production of perithecia of *Venturia pyrina* Aderh. Sprayed and unsprayed leaves were overwintered and examined for perithecia by the methods used in the similar studies on apple scab. The results, which will not be given in detail, were closely similar to those obtained in the apple scab work.

At Hood River, Oreg.[9], in 1934–35, certain copper-lime-arsenic mixtures were applied to branches of three large Anjou pear trees bearing twig lesions caused by *Venturia pyrina*. The same treatments were given on two dates, December 5 and March 18, so that one series of branches received only the earlier application, another only the later, and a third received both. Unsprayed branches served as controls. Because of circumstances beyond the control of the experimenters, these trees also received a dormant application of lime-sulphur.

Samples of twigs collected May 12 were sent to Madison, where they were examined microscopically for the percentage of lesions that bore conidia of the scab fungus. The results, which will not be given in detail, indicated that the more efficient treatments had reduced the percentage of sporulating lesions by approximately three-fourths

[8] Acknowledgment is made to Dr. W. D. Valleau of the Kentucky Agricultural Experiment Station for his kind cooperation in this experiment and permission to report the results.

[9] Acknowledgment is made to L. Childs of the Hood River Branch Experiment Station of Oregon and J. Kienholz of the Office of Horticultural Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture, for their kind cooperation in this experiment and permission to report the results.

to nine-tenths. However, under the conditions of the experiment, there was more spray injury than could be tolerated commercially.

While the host injury caused in this experiment was distinctly discouraging, it should be noted that all the formulas used were comparatively low in lime. The fact that the conidial inoculum appeared to be substantially reduced by the formulas tested in this initial trial suggests possibilities of its more adequate suppression by better adapted sprays.

CHERRY LEAF SPOT

At Madison in 1933, branches of sour cherry (*Prunus cerasus* L.) abundantly affected by leaf spot, caused by *Coccomyces hiemalis* Higgins, were given fall applications of certain copper-lime-arsenic mixtures. Sprayed and unsprayed leaves were overwintered and examined for apothecia in the spring by the methods used in the similar studies on apple scab. The results, which will not be given in detail, were closely similar to those obtained in the studies on apple and pear scab.

BLACK KNOT OF CHOKECHERRY

In three series of experiments at Madison in 1933, certain sprays were applied by atomizers to cut twigs of chokecherry (*Prunus virginiana* L.) bearing lesions of black knot, caused by *Dibotryon morbosum* (Schw.) T. & S., to test their effectiveness in suppressing the production of ascospores of this pathogen. Two to ten twigs bearing living knots with abundant ascocarp initials or ascocarps were used per treatment. The cut ends of the twigs were kept in water, which was changed frequently. A summary of the treatments and results appears in table 8.

TABLE 8.—*Results of spraying cut twigs of chokecherry for the suppression of the ascigerous stage of the black knot fungus, Madison, Wis., 1933*

Series and date of application	Formula [1]	Ascospores present at stated dates	
Series 1, Feb. 1	1. Untreated	Yes	Feb. 15
	2. CS1+L (neut.)[2]+FO1¼	Yes	
	3. CS1+L (neut.)+Cai1+FO1½	No	
	4. CS1+ L1+Cai1+FO3¼	No	
	5. CS1+L 1+Cai½+FO⅝	No	
	6. CS¾+L ½+Cai½+FO⅜	No	
Series 2, Feb. 16	7. Untreated	Yes	Feb. 25
	8. CS1+L ½+FO⅜	Yes	
	9. CS1+L (neut.)+FO1¼	Yes	
	10. CS1+L 1+Cai¾+FO¾	No	
	11. CS1+L 1+Cai ½+FO⅝	No	
	12. CS1+L 1+Cai¼+FO1½	No	
	13. CS1+L ½+Cai½+FO1½	No	
	14. CS1+L ½+Cai¼+FO⅜	No	
Series 3, Mar. 9	15. Untreated	Yes	Mar. 14
	16. CS1+L ½+Cai½+FO1½	No	

[1] For explanation of symbols, see footnote 1 of table 2.
[2] Milk of lime was added in successive small amounts until the supernatant liquid, after agitation, no longer gave a positive potassium ferrocyanide test for copper.

On February 1, when the first series of sprays was applied, the asci of the black knot fungus were in an early stage of development. A few spores had been delimited, but none were mature. The twigs, after drying overnight in the laboratory, were wet with distilled water and held in a moist chamber for 1 week at 7° and for another at 12° C.

Abundant ascospores were developed on the unsprayed knots, and the ascocarps from this source were in good condition when examined on February 15. Abundant ascospores were also matured on the cankers sprayed with neutral bordeaux, but none on those sprayed with the copper-lime-arsenic mixtures. No asci were found in the material that received formula 4. Abundant asci were observed in the material that received formulas 3, 5, and 6, respectively; but they were abnormal, appeared to be degenerating, and bore no delimited spores. Fragments of knots from each treatment were moistened and placed in Petri dishes above agar for a test on spore discharge. Abundant ascospores were discharged from the untreated fragments. Discharge from the material that received the bordeaux treatment was abundant, but apparently less than from the untreated. No discharge occurred from fragments that had been treated with any of the copper-lime-arsenic mixtures.

The second series of sprays was applied February 16. The twigs, after drying overnight in the laboratory, were moistened and held in a moist chamber for 9 days at 16° C. The results of microscopic examinations and a test of ascospore discharge were similar to those of the first series. Abundant ascospores were developed on the untreated knots. Bordeaux failed to prevent abundant ascospore development and discharge. No mature ascospores were obtained from any of the knots that received the copper-lime-arsenic treatments. Figure 7, *E*, *F*, illustrates the condition of a typical perithecium that received treatment 12, in contrast with that of a normal ascocarp from an untreated lesion.

On March 9, when the third series was started, many of the ascocarps contained mature asci, and discharged ascospores freely when moistened. After the spray was applied the knots were allowed to dry out of doors. They were then wet with distilled water and placed in a moist chamber for 4 days at 16° C. On March 13 the unsprayed perithecia were normal in appearance, contained mature asci in abundance, and discharged ascospores freely. The asci in the treated ascocarps had degenerated, and no ascospores were discharged when fragments of the treated knots were moistened and placed over agar in Petri dishes.

THREAD BLIGHT OF FIG[10]

At Union, La., fig (*Ficus carica* L.) branches bearing abundant sclerotia of the thread blight fungus, *Corticium koleroga* (Cke.) v. Höhn. were sprayed January 25, 26 and again February 27, 28, 1935, with each bordeaux mixture (8–8–50 in the first application, 4–4–50 in the second) and certain copper-lime-arsenic formulas, respectively. The bordeaux treatments considerably reduced the amount of infection that developed in the following season, but did not satisfactorily control the disease. The two treatments with one of the copper-lime-arsenic preparations (formula 209 of table 2) gave efficient control. The disease developed abundantly on the unsprayed branches that served as checks. No spray injury was observed.

In 1936 the experiment was repeated with modifications. Two trees, about 12 years old and bearing abundant sclerotia, mycelia, and

[10] Acknowledgment is made to Dr. E. C. Tims and P. J. Mills, of the Louisiana Agricultural Experiment Station, for permission to make the following statement regarding their work on this disease.

basidiospores of the thread blight fungus, were sprayed January 9 and again February 26 with two copper-lime-arsenic formulas. Formula 108 of table 2 was used in both applications on tree no. 1, and formula 142 (with fish oil, one-half of 1 percent) of the same table was similarly applied to three-fourths of tree no. 2. The remainder of tree no. 2 served as a check. The disease was completely controlled on tree no. 1. A trace of infection (on two twigs) occurred on the sprayed portion of tree no. 2. The disease developed abundantly on the unsprayed part of tree no. 2. No spray injury was observed.

SEED TREATMENT EXPERIMENTS

The high eradicant fungicidal efficiency of copper-lime-arsenic mixtures and the ease with which their toxic properties can be varied through a wide range of control by modifications of formula suggested tests of their potentialities for use in seed treatments. The fact that these mixtures are capable of exercising toxic effects in an alkaline, neutral, or acid medium by means of soluble materials that diffuse from the spray or dust residues (*26*) suggested that they might find adaptation against seed- and soil-borne pathogens. About 200 dusts of good physical properties were prepared, usually by filtering the liquid preparations, drying the residues, and grinding in a mortar. The experimental work was centered about wheat (*Triticum aestivum* L.) bunt, caused by *Tilletia tritici* (Bjerk.) Wint. and *T. levis* Kühn. In greenhouse experiments that have not yet been reported, certain of these copper-lime-arsenic dusts were somewhat more efficient than commercial copper carbonate or ethyl mercury phosphate dusts in controlling this disease in infested soil. In these tests, however, the copper-lime-arsenic dusts used were not capable of satisfactorily controlling bunt in cases of severe outbreaks resulting from a soil-borne inoculum. Dusts of the type under discussion clearly have substantial value for seed-treatment purposes. It remains for further investigation to determine whether they can advantageously be adapted to practical use.

DISCUSSION

The results of these studies as they relate to apple scab control have been discussed in an earlier section. It remains to consider them in relation to potentialities of increased use of eradicant chemical measures in the control of other diseases. This discussion will be introduced by the following brief survey of pertinent literature.

The first fungicides to find an important adaptation to plant disease control were of the eradicant type, and compounds of copper and arsenic, respectively, were among the first materials to be used successfully for this purpose. In his classic memoir on bunt of wheat, Prévost (*27*) reported experimental demonstration of the lethal action of copper sulphate and various other chemical agents on the spores of the bunt pathogen, and described a well-founded method for large-scale use of a copper sulphate solution for seed treatment. He reviewed previous work on seed treatments for bunt control, giving special attention to arsenous oxide, which had been used in England, and reporting it to be much inferior to copper sulphate in fungicidal action and other important qualities.

Washing dormant grapevines with a solution of iron sulphate and sulphuric acid to combat anthracnose was one of the earliest successful

adaptations of liquid fungicides to the treatment of plants for disease control (*29*). It was an eradicant procedure, and, if it had received more consideration, might have pointed the way to further substantial developments in this direction. However, the discovery of bordeaux mixture and the great development of dominantly protectant spraying that followed appear to have overshadowed this promising beginning in the evolution of methods for suppression of the primary inoculum of phytopathogenic fungi by eradicant chemical treatments.

The dominance of protectant spraying in the control of orchard diseases since the discovery of bordeaux mixture, the great achievements and present importance of protectant spraying, and the current status of eradicant measures in orchard disease control have been discussed by one of the writers (*15*).

In the rapid expansion of spraying methods that followed the introduction of bordeaux mixture, various treatments of dormant plants were tried more or less empirically against many diseases in the hope of suppressing the pathogens. In most cases, however, the results did not justify the treatments. While eradicant spraying has come into very successful use against certain diseases (e. g., peach leaf curl, caused by *Taphrina deformans* (Berk.) Tul.), it has continued to receive comparatively little attention.

Although arsenic is not usually regarded as an active fungicidal element, it has long been known that various arsenical compounds are toxic to fungi (*22*). The early work with arsenous oxide in seed treatments against bunt of wheat (*27*) has been mentioned. Knowles (*21*) reports an early attempt to combat dry rot of ships by the use of arsenical materials. Reference may be made to Horton and Salmon (*11*) and McCallan and Wilcoxon (*22*) for surveys of more recent literature on the fungicidal action of arsenical compounds. Of special interest in relation to the present paper are the work of Curtin (*2*) and Curtin and Thordarson (*3*) on the use of arsenites in wood preservation, that of Viala (*30*) and others on control of "esca" or apoplexy of the vine by a dormant application of an arsenical spray (chiefly sodium arsenite), and various experiments on the use of arsenical compounds in seed treatments (*22*) and in spraying plants in foliage (*5, 7, 8, 9, 11, 28*) to combat certain diseases. McCallan and Wilcoxon (*22*) report that in their experiments on spore germination arsenic was toxic as arsenous acid, sodium arsenate ($Na_2HAsO_4.7H_2O$), and elementary powdered arsenic. They state that, in conformity with the results of Wollenweber (*34*), their data indicate "a great variation in sensitivity to arsenic among fungi", and suggest that, while arsenic may find increased use for fungicidal purposes, its adaptation will probably be confined to certain specific cases, such as they have noted. Working with a different technique, Curtin and Thordarson (*3*) found that zinc meta-arsenite was highly and rather uniformly toxic to the 14 species of fungi that they studied, and Palmiter and Keitt (*26*) report that calcium arsenites were highly toxic to all of the 8 species of phytopathogenic fungi included in their studies.

It seems unnecessary for the purposes of the present paper to discuss the rather extensive literature (*32*) dealing with the injurious or beneficial effects of arsenical compounds on seed plants, the accumulation of arsenic in the soil, its inactivation in the soil by chemical

reactions, its liberation from the soil in a gaseous state as a result of the activities of micro-organisms, and related subjects.

Additions of various arsenical compounds to bordeaux mixture for spraying plants in foliage have long been made for the purpose of insect control. Formerly, arsenites or materials containing them, such as paris green and london purple, were commonly used; but, in later years, these have largely been replaced by arsenates. It has long been recognized that the addition of these arsenical compounds may increase the fungicidal value of sprays. However, at the concentrations used in practice, such preparations have comparatively little eradicant fungicidal value, and are unsuited to the purposes of the present investigation.

The results that have been presented in the present paper and those of a correlated study on the toxicity of copper-lime-arsenic mixtures to certain phytophathogenic fungi, using toximetric cultural methods (*26*), show that suitable copper-lime-arsenic mixtures have powerful eradicant fungicidal properties, and that the toxicity of preparations of this type can be varied through a wide range by choice of the arsenical component, modifications in the amounts and proportions of the ingredients, and the use of amendments. Under the conditions employed in the toximetric experiments (*26*) the calcium arsenites studied showed a higher toxicity to the test fungi than copper sulphate, and the copper-lime-arsenite mixtures commonly showed a toxicity equal to or greater than the summation of the toxicities of their separate ingredients. Furthermore, it was shown that copper-lime-arsenic preparations liberate soluble materials that are capable of diffusing through an acid, neutral, or alkaline medium and exercising fungicidal action at considerable distances from the undissolved residues. Under like conditions, the copper-lime component (bordeaux mixture), when used alone, exercised little or no toxic action by means of diffused materials when the medium was alkaline or neutral, and very little in the acid range tested. These facts aid in explaining the great superiority of suitable copper-lime-arsenic preparations over bordeaux mixture in eradicant fungicidal power.

The studies reported herein show further that suitable copper-lime-arsenic mixtures were usually highly effective in either preventing sporulation of the various pathogens against which they were tested or else in killing or inactivating the spores before they were disseminated.

The dictum that a phytopathogenic fungus cannot be effectively combatted by spraying after it has become well established in the host tissues is rather widely accepted. Attention is invited to the fact that most pathogenic fungi that grow on or in plant tissues sporulate at or near the surface, or else their spores pass to the surface before they can be disseminated. The production and liberation of the primary inoculum is therefore to be regarded as a potentially vulnerable stage in the life history of many phytopathogenic fungi, and one against which surface treatments with suitable eradicant fungicides would seem to have much greater possibilities than have hitherto been generally recognized.

The studies thus far undertaken have been exploratory and comparatively limited. Much additional work remains to be done if eradicant chemical measures of the type under consideration are to find substantially increased practical adaptation in programs of soundly founded complementary procedures for the control of plant

diseases now combatted chiefly by protectant fungicides. The work reported herein was centered about copper-lime-arsenic preparations only because these seemed to be much the most promising materials tried. Further studies directed toward the development of better adapted materials seem desirable. Only a small beginning has been made on experimental studies of the effects of eradicant treatments, and the consequent limitation of primary inoculum, on epidemiology and control of the diseases concerned. Additional work in this undeveloped field and in the underlying fundamentals of epidemiology is highly essential to the evolution of soundly based control programs. Attempts at practical applications of the methods under investigation await further advances in studies of the eradicant methods and their effects.

The present methods of protectant spraying are the result of a prodigious amount of investigation and experience in many lands throughout a 50-year period. The problems of chemical eradication seem to be no less difficult than those of chemical protection. If the use of eradicant measures to complement protectant spraying is to be substantially advanced it will be only as the result of adequate experimentation. It is hoped that the evidence now available on the potentialities of eradicant chemical treatments for plant disease control may justify other workers in undertaking studies in this comparatively neglected field.

SUMMARY

Accomplishments and limitations of plant disease control by protectant spraying are discussed, and attention is called to the need for increased development of control programs of complementary procedures based on the principles of immunization and eradication as well as protection. A report is given of studies of potentialities for increased use of eradicant chemical measures for direct attack upon the pathogen, with the aim of reducing it to a survival level at which it can be more surely and economically controlled.

The studies on apple (*Malus sylvestris* Mill.) scab, caused by *Venturia inaequalis* (Cke.) Wint., were projected along three major lines: (1) Materials and methods for chemical eradication, (2) effects of eradicant procedures on epidemiology and control, and (3) large-scale orchard tests of the feasibility of adapting eradicant chemical measures to the practical control of the disease.

In small-scale experiments many materials were tested in a single spray treatment after harvest and before many leaves had fallen, with the aim of suppressing the ascosporic inoculum. Various mixtures of aqueous solution of copper sulphate, milk of lime, and certain arsenical compounds (chiefly calcium and zinc arsenites) were highly effective, often completely preventing production of ascospores in individual tests. The results from over 200 formulas are reported and discussed. Many of these mixtures caused severe host injury. Others showed high fungicidal effectiveness without having thus far caused serious injury to the host. Further studies are necessary to determine which formulas are best and whether they are safe enough for use in orchard practice.

In two seasons the epidemiology of apple scab was studied comparatively in two small orchards about 100 yards apart, one sprayed with copper-lime-arsenic mixtures in the fall and the other unsprayed.

Neither received summer sprays. Under the conditions encountered, scab development in the orchard that received the fall spray was strikingly retarded and reduced. Certain confirmatory orchard observations are reported.

A spring treatment of the fallen leaves with an ammonium sulphate solution killed the ascocarps of *Venturia inaequalis*. Other similar experiments are in progress.

The studies on apple scab, which are being continued, have not reached a stage to warrant large-scale trials on the feasibility of adapting the eradicant chemical methods here discussed to practical use in controlling this disease.

Small-scale exploratory tests of the potentialities of copper-lime-arsenic mixtures for suppressing primary inocula were made in relation to eight other phytopathogenic fungi. Fall spraying of pear (*Pyrus communis*) and cherry (*Prunus cerasus*) leaves infected, respectively, by *Venturia pyrina* and *Coccomyces hiemalis*, and dormant spraying of chokecherry (*Prunus virginiana*) and raspberry (*Rubus strigosus*) infected, respectively, by *Dibotryon morbosum* and *Mycosphaerella rubina* were highly effective in suppressing production of ascospores. Dormant spraying was highly effective in suppressing production of conidia of *Sclerotinia fructicola* on overwintered persistent mummified fruits of plum (*Prunus domestica*), and partly effective in suppressing production of pycnospores of *Phyllosticta solitaria* on apple and conidia of *Cladosporium carpophilum* and *Venturia pyrina* on peach (*Amygdalus persica*) and pear, respectively. It was highly effective against sclerotia, mycelia, and basidiospores of *Corticium koleroga*, appearing to give satisfactory control of the thread blight of fig (*Ficus carica*). In greenhouse trials in infested soil, copper-lime-arsenic dusts gave somewhat better control of wheat (*Triticum aestivum* Host) bunt, caused by *Tilletia tritici* and *T. levis*, than did commercial copper carbonate or ethyl mercury phosphate dusts.

The studies reported herein show: (1) That the toxicity of copper-lime-arsenic mixtures can be varied through a wide range of control by choice of the arsenical component, modifications in the amounts and proportions of the ingredients, and the use of amendments; (2) that these preparations liberate soluble materials that are capable of diffusing through an acid, neutral, or alkaline medium and exercising fungicidal action at considerable distances from the undissolved residues; (3) that suitable mixtures are highly effective against fungal fruiting structures at the surface, or near a permeable surface, of invaded tissues; and (4) that many phytopathogenic fungi are potentially vulnerable to attack by surface applications of eradicant fungicides.

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THE TOXICITY OF COPPER-LIME-ARSENIC MIXTURES TO CERTAIN PHYTOPATHOGENIC FUNGI GROWN ON MALT AGAR PLATES [1]

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INTRODUCTION

The work reported herein is part of a series of studies of potentialities for increased application of the principle of eradication to the control of certain types of plant disease through the use of fungicides. Keitt and associates (*2*, *3*, *5*, *6*)[2] have shown by means of spraying experiments that various copper-lime-arsenic mixtures possess strong eradicant fungicidal properties. The present paper reports the results of a comparative study of the toxicity of some of these mixtures and of their separate ingredients to certain representative phytopathogenic fungi grown in vitro (*4*). While it is recognized that such work cannot take the place of field experiments, the greater speed of operations and more accurate control of environmental conditions in the laboratory facilitate certain types of experimentation that materially assist in guiding the field work and interpreting its results.

TOXIMETRIC STUDIES

METHODS

The technique employed in this study is based on the widely used method of Schmitz et al. (*8*) for toximetric studies of wood preservatives.

The toxic materials were dissolved or suspended in water in 250-cubic centimeter flasks at twice the concentration desired in the toxic medium, and steamed at atmospheric pressure for 1 hour. When two or more chemicals entered into the toxic material, they were steamed separately in appropriate volumes of water. The nutrient medium (agar, 34 grams; Trommer's extract of malt, 50 grams; water, 1,000 cubic centimeters) was freshly prepared at twice the concentration used in the tests, and suitable quantities were autoclaved at 15 pounds' pressure for 30 minutes. After partial cooling, equal volumes of the medium and the solution or suspension of toxic material were mixed. When two or more chemicals entered into the toxic material they were introduced into the mixing flask in the following order: Copper or zinc sulphate, lime, the arsenical compound. The nutrient medium was added last, except in a few early experiments in which it was used to dilute the lime component. The results from these two procedures for adding the nutrient medium were so closely similar that they do not require separate treatment. The mixtures were shaken as each material was added. After thorough agitation to insure uniform suspension of solid materials, each mixture of agar and test material was

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[2] Reference is made by number (italic) to Literature Cited, p. 451.

poured in amounts of approximately 25 cubic centimeters into Petri plates, where after further agitation it was allowed to solidify. The temperature was regulated so that the toxic medium solidified very quickly after the plates were poured.

The mycelium of the test fungi for planting the plates was cut in disks 5 millimeters in diameter from malt-agar plate cultures of suitable age (5 to 10 days) to have developed a vigorous growth. Two plantings were made on each plate of toxic medium on a median line, each disk being about equally distant from the center and the edge of the plate. Control plates containing the suitably diluted medium, but no toxic material, were similarly planted in each test.

The plates were incubated in darkness at 20° C. for 2 weeks when the faster growing fungi, *Sclerotinia fructicola* (Wint.) Rehm. *Glomerella cingulata* (Stoneman) Sp. and con S., *Phyllosticta solitaria* E. and E., and *Physalospora obtusa* (Schw.) Cke., were used, and for 3 weeks when *Venturia inaequalis* (Cke.) Wint., *V. pyrina* Aderh., *Elsinoë veneta* (Burk.) Jenk., and *Cladosporium carpophilum* Thum. were the test organisms. If no growth appeared during this time the disks were transferred to malt agar slants, and if then no growth appeared after a period equal to that on the toxic medium the mycelium in the disks was considered dead.

Except as otherwise stated, the results are summarized (tables 1, 2, 3, 4) in terms of (1) the highest percentage concentration by weight of the toxic substance that in any case allowed growth of the test fungus on the plate, and (2) the lowest concentration that always killed the mycelium. At least two tests were made with each organism on each medium. Each test usually consisted of duplicate plates, with a total of four colonies, though as many as 5 plates and 10 colonies have been used. In general, the results of the replications agreed closely, though, as might be expected, those from some of the less toxic materials (e. g., diacalcium arsenate) showed more variation. Usually the concentration of the toxic materials was increased until the killing point was determined. However, some of the test materials did not kill at concentrations of 2 or 3 percent; and, since it is ordinarily impracticable to use such high dosages for spraying, the actual killing point of these preparations was not determined.

MATERIAL

Source and Purity of the Chemical Materials Used

The arsenical compounds used in this study, which were finely ground products obtained from several commercial companies, were analyzed[3] according to the methods of the Association of Official Agricultural Chemists (*1*). Results appear in the following paragraphs. The percent of moisture is based on the original air-dry samples. The water-soluble and total arsenic are expressed as percent As_2O_3 for the arsenites and As_2O_5 for the arsenates, based on the oven-dry (110° C.) samples. There was not sufficient difference in the results from different lots of the same compound to warrant distinctive designation of each lot in relation to the analyses.

Calcium arsenites.—While the calcium arsenites are designated as mono-, di-, and tri-, it is recognized that each probably contained

[3] Grateful acknowledgments are made to Prof. V. W. Meloche for supervising and to C. N. Clayton for making these determinations.

some admixture of the others. Each of the three samples of monocalcium arsenite used contained less than 1 percent of moisture. The water-soluble arsenic ranged from 23 to 28 percent, and the total arsenic from 70 to 74 percent. The two samples of dicalcium arsenite showed 2.7 and 4.5 percent of moisture, 8 and 15 percent of water-soluble arsenic, and 59 and 61 percent of total arsenic, respectively. The two samples of tricalcium arsenite showed 1.0 and 1.6 percent of moisture, 1.7 and 9.0 percent of water-soluble arsenic, and 55 and 54 percent of total arsenic, respectively.

Zinc arsenite.—All the zinc arsenite was obtained from the same source. It contained 0.4 percent of moisture, 0.6 percent of water-soluble arsenic, and 42 percent of total arsenic.

Copper arsenite.—The copper arsenite (c. p.), which was reported by the manufacturer to be in the cupric form, contained 2.2 percent of moisture, 2.0 percent of water-soluble arsenic, and 41 percent of total arsenic.

Paris green.—The paris green, which came from one source, contained 0.3 percent of moisture, 0.2 percent of water-soluble arsenic, and 57 percent of total arsenic.

Iron arsenite.—The iron arsenite (c. p.), which was reported by the manufacturer to be in the ferrous form, contained 5.6 percent of moisture, 3.0 percent of water-soluble arsenic, and 42 percent of total arsenic.

Calcium arsenates.—The dicalcium arsenate used contained 0.6 percent of moisture, 10 percent of water-soluble arsenic, and 54 percent of total arsenic as As_2O_5. The tricalcium arsenate used was not available when the arsenic determinations were made.

Other chemical materials.—Crystalline cupric sulphate ($CuSO_4.5H_2O$) of technical grade, c. p. crystalline zinc sulphate ($ZnSO_4.6H_2O$), and lump calcium oxide of U. S. P. grade were used.

Source of Test Fungi

The test fungi were obtained as follows: *Venturia inaequalis*, six monoconidial isolates (15, 17, 18, 22, 24, and 30) used and described by Palmiter (7); *V. pyrina*, a monoconidial isolate from a pear leaf at Madison, Wis.; *Cladosporium carpophilum*, a monoconidial isolate from peach twigs at Madison; *Phyllosticta solitaria*, isolated from apples from Indiana; *Elsinoë veneta*, from raspberry canes at Madison; *Glomerella cingulata*, a culture from H. W. Anderson, Urbana, Ill.; *Physalospora obtusa*, a monoconidial isolate from H. H. Foster, Madison; and *Sclerotinia fructicola*, a monoconidial isolate from cherry fruit at Sturgeon Bay, Wis.

EXPERIMENTAL RESULTS

TOXICITY OF THE SEPARATE INGREDIENTS OF CERTAIN COPPER-LIME-ARSENIC MIXTURES

Data on the toxicity of six arsenites, copper sulphate, and calcium oxide, respectively, to each of eight test fungi are shown in table 1. The toxicity of the arsenites varied through a wide range. The monocalcium preparation was the most toxic. The tricalcium arsenite was slightly less toxic. Paris green was less toxic than the calcium arsenites, but more toxic than copper arsenite. The zinc and iron

preparations were the least toxic of the arsenites tested, neither of them killing any of the test fungi except *Physalospora obtusa* at 2 percent, the highest concentration used.

The concentration of copper sulphate required to kill ranged from 3 to more than 10 times the lethal concentration of monocalcium arsenite, depending on the test fungus used.

The toxicity of calcium oxide varied much with the test fungi, one being killed at a concentration of 0.4 percent while three were able to withstand 2 percent.

No two test fungi reacted alike. *Physalospora obtusa* was the most sensitive, being killed by all the test materials used at concentrations less than 2 percent. *Glomerella cingulata* was the most resistant to copper sulphate, while *Sclerotinia fructicola* was the most resistant to the arsenites. Figure 1 shows the growth made by *Physalospora obtusa* on malt agar plates containing graduated concentrations of each of four different arsenites and of copper sulphate.

TABLE 1.—*Toxicity of the separate ingredients of certain copper-lime-arsenic mixtures to eight phytopathogenic fungi*

Toxic agent	Criteria	Critical concentrations [1] for—							
		Venturia inaequalis 17	*Venturia pyrina*	*Cladosporium carpophilum*	*Phyllosticta solitaria*	*Elsinoë veneta*	*Glomerella cingulata*	*Physalospora obtusa*	*Sclerotinia fructicola*
		Percent	Percent	Percent	Percent	Percent	Percent	Percent	Percent
Monocalcium arsenite	Growth	0.08	0.06	0.04	0.02	0.08	0.06	0.02	0.06
	Death	.10	.10	.06	.06	.10	.10	.04	.12
Tricalcium arsenite	Growth	.08	.08	.04	.02	.06	.06	.04	.06
	Death	.12	.12	.06	.08	.08	.10	.06	.14
Zinc arsenite	Growth	>2.00	>2.00	>2.00	>2.00	>2.00	>2.00	1.00	>2.00
	Death							1.50	
Iron arsenite	Growth	>2.00	>2.00	>2.00	>2.00	>2.00	>2.00	.40	>2.00
	Death							.50	
Copper arsenite	Growth	>2.00	>2.00	.50	.20	>2.00	1.00	.10	>2.00
	Death			1.00	>2.00		>2.00	.50	
Paris green	Growth	.50	>2.00	.10	.05	.50	.50	.05	.20
	Death	.60		.60	.20	2.00	.60	.10	1.00
Copper sulphate	Growth	.10	.20	.10	.15	.20	.30	.10	.80
	Death	.30	.45	.60	.20	.70	2.00	.60	1.00
Calcium oxide	Growth	.40	.40	.50	.30	.30	1.00	.40	.60
	Death	.80	.50	>2.00	1.00	.40	>2.00	.50	>2.00

[1] The figure shown for growth is the highest concentration tested at which growth occurred in any experiment; for death, the lowest that always killed. > indicates that the critical concentration was greater than the figure shown.

Data on the relative toxicities of each of four arsenites, two arsenates, copper sulphate, and calcium oxide to each of six monoconidia isolates of *Venturia inaequalis* are shown in table 2. The three calcium arsenites gave similar results and were consistently more toxic than copper sulphate. The tricalcium arsenate was the least toxic compound tested in this experiment, all of the isolates being able to grow at the highest concentrations used. The dicalcium arsenate was only slightly more toxic. The toxicity of paris green was intermediate between that of the calcium arsenites and the arsenates.

As pointed out by Palmiter (7), different isolates of *Venturia inaequalis* may show considerable variation in tolerance to toxic materials in the medium on which they are grown. Isolates 22 and 30 were more resistant to killing by the calcium arsenites than were the others. Isolates 22 and 17 were the more resistant ones to copper sulphate.

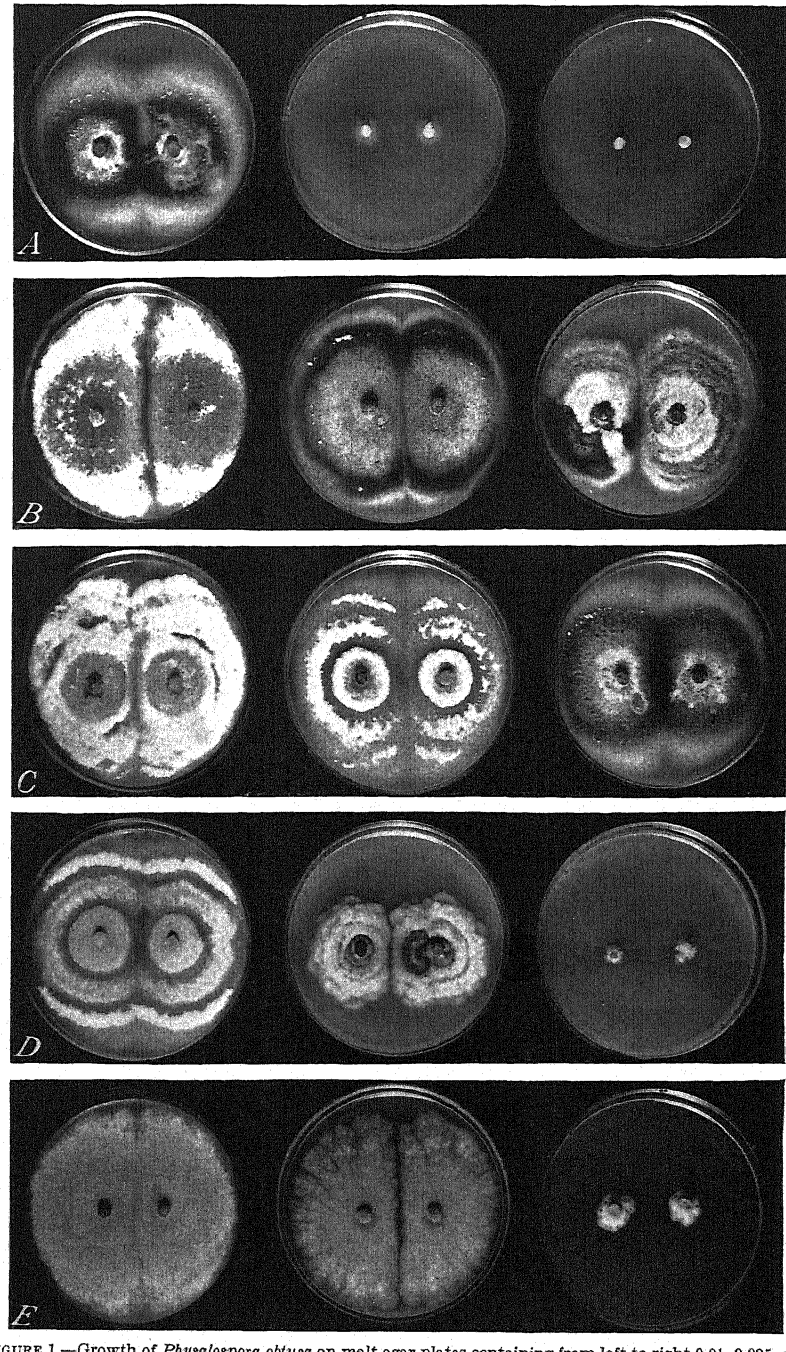


FIGURE 1.—Growth of *Physalospora obtusa* on malt agar plates containing from left to right 0.01, 0.025, and 0.05 percent, respectively, of: *A*, Monocalcium arsenite; *B*, zinc arsenite; *C*, iron arsenite; *D*, copper arsenite; *E*, copper sulphate.

TOXICITY OF CERTAIN COPPER-LIME-ARSENIC MIXTURES

Data on the toxicity of certain copper-lime-arsenic mixtures and of their copper-lime component, used alone, are shown in table 3. The copper-lime-monocalcium arsenite preparation was the most toxic of the mixtures studied, *Sclerotina fructicola* being the only test fungus that was not killed by 10 percent of the reference concentration. The similar tricalcium arsenite preparation was distinctly less toxic. The paris green mixture was more toxic than the tricalcium arsenite preparation against certain test fungi and less toxic against others. In all cases it was more toxic than the zinc, iron, or copper arsenite mixtures. The copper-lime component, used alone, was relatively low in toxicity, most of the test fungi surviving at the highest concentration tried.

TABLE 2.—*Toxicity of the separate ingredients of certain copper-lime-arsenic mixtures to 6 isolates of Venturia inaequalis*

Toxic agent	Criteria	Critical concentrations [1] for isolate—					
		15	17	18	22	24	30
		Percent	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
Monocalcium arsenite	Growth	0.08	0.08	0.08	0.12	0.08	0.10
	Death	.12	.12	.12	.18	.12	.14
Dicalcium arsenite	Growth	.08	.08	.08	.10	.08	.08
	Death	.12	.12	.10	.12	.12	.14
Tricalcium arsenite	Growth	.08	.08	.10	.10	.08	.08
	Death	.10	.12	.12	.16	.12	.12
Paris green	Growth	.30	.50	.30	.50	.30	.30
	Death	.50	.60	>.30	1.56	>.30	>.30
Dicalcium arsenate	Growth	1.00	.60	1.00	1.80	1.00	.50
	Death	3.00	>3.00	>2.00	>3.00	>2.00	>2.00
Tricalcium arsenate	Growth	>3.00	>3.00	>2.00	>3.00	>2.00	
	Death						
Copper sulphate	Growth	.08	.10	.12	.12	.10	.14
	Death	.14	.30	.18	.40	.16	.18
Calcium oxide	Growth	>.50	.40	>.50	>.50	>.50	.10
	Death		.80				.50

[1] See footnote 1, table 1.

TABLE 3.—*Toxicity of certain copper-lime-arsenic mixtures to 8 phytopathogenic fungi*

Formulas at reference concentrations [1] in grams per 100 cubic centimeters	Criteria	Critical concentrations [1 2] of reference formulas for—							
		Venturia inaequalis 17	*Venturia pyrina*	*Cladosporium carpophilum*	*Phyllosticta solitaria*	*Elsinoë veneta*	*Glomerella cingulata*	*Physalospora obtusa*	*Sclerotinia fructicola*
		Percent	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
A [3]	Growth	10	20	10	10	10	>100	20	20
	Death	100	>100	>100	100	>100	>100	>100	>100
A+monocalcium arsenite, 0.5	Growth	5	5	2	5	5	5	2	5
	Death	10	10	10	10	10	10	5	20
A+tricalcium arsenite, 0.5	Growth	5	10	5	5	5	5	2	10
	Death	>10	>100	40	10	10	100	5	>100
A+zinc arsenite, 1.0	Growth	20	20	10	10	10	10	10	10
	Death	>100	>100	>100	60	>100	>100	40	>100
A+iron arsenite, 0.5	Growth	60	60	5	5	20	10	5	20
	Death	>100	>100	100	100	100	>100	20	>100
A+copper arsenite, 0.5	Growth	60	5	5	5	5	10	5	10
	Death	100	>100	100	60	60	>100	20	>100
A+paris green	Growth	10	10	5	5	5	5	5	5
	Death	60	60	60	10	20	40	10	>100

[1] The formulas are listed at reference concentrations, such as were used in spraying experiments, and the critical concentrations shown are expressed in percentages of these reference formulas.
[2] See footnote 1, table 1.
[3] A=the copper-lime component: $CuSO_4 \cdot 5H_2O$, 0.75 grams, plus calcium oxide, 0.5 grams, per 100 cubic centimeters.

In tests with *Venturia inaequalis* (table 4), the copper-lime-monocalcium arsenite mixture was more toxic than the similar dicalcium and tricalcium arsenite preparations, and equally toxic to all six isolates used. The similar dicalcium and tricalcium arsenate mixtures showed little increase in toxicity over the preparation containing only the copper sulphate and lime. When zinc sulphate was substituted for the copper sulphate, a slight decrease in toxicity resulted.

Whereas the several isolates of *Venturia inaequalis* differed considerably from one another in their reactions to the separate ingredients of the copper-lime-arsenic mixtures (table 2), they were rather uniform in their response to the mixtures (table 4).

The failure of certain fungi to grow on media containing comparatively high concentrations of lime suggested a study of the tolerance of high alkalinity by the test organisms. Experiments were therefore performed to determine the optimal pH value for growth of the test fungi and the upper pH limits at which they could be expected to make satisfactory growth for these tests. Varying amounts of sodium hydroxide were added to the malt extract agar to give the desired range of pH. On the basis of diameter of colony, *Glomerella cingulata* and *Elsinoe veneta* made their best growth when the initial pH value of the medium was 8.0 to 8.7, while the other six fungi grew best when the medium was adjusted in the range of pH 5.3 to 6.7. All eight fungi were able to make satisfactory growth for these tests when the medium had an initial pH value of 8.5.

When the ingredients of the mixtures were used separately in the medium all the toxic media except those made with calcium oxide gave pH readings below 8.5. The medium containing 0.5 percent of the calcium oxide, used alone, was too alkaline for satisfactory growth of the test fungi.

TABLE 4.—*Toxicity of certain copper- or zinc-lime-arsenic mixtures to 6 isolates of Venturia inaequalis*

Formulas at reference concentrations[1] in grams per 100 cubic centimeters	Criteria	Critical concentrations [1] [2] of reference formulas for isolate—					
		15	17	18	22	24	30
		Percent	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
A[3]	Growth	20	10	10	20	20	20
	Death	>100	100	>100	>100	>100	>100
A+monocalcium arsenite, 0.5	Growth	5	5	5	5	5	5
	Death	10	10	10	10	10	10
A+dicalcium arsenite, 0.5	Growth	5	5	5	5	5	5
	Death	10	10	>10	>10	>10	>10
A+tricalcium arsenite, 0.5	Growth	5	5	5	5	5	5
	Death	10	>10	10	>10	>10	>10
A+dicalcium arsenate, 1.0	Growth	5	5	-------	10	-------	-------
	Death	>100	>100	-------	>100	-------	-------
A+tricalcium arsenate, 1.0	Growth	10	5	-------	10	-------	-------
	Death	100	100	-------	100	-------	-------
B[4]	Growth	20	100	20	5	20	100
	Death	>100	>100	>100	>100	100	-------
B+tricalcium arsenite, 0.5	Growth	5	5	5	5	5	5
	Death	10	>10	>10	>10	>10	>10

[1] The formulas are listed at reference concentrations, such as were used in spraying experiments, and the critical concentrations shown are expressed in percentages of these reference formulas.

[2] See footnote 1, table 1.

[3] A=the copper-lime component: $CuSO_4.5H_2O$, 0.75 gram, plus calcium oxide, 0.5 gram, per 100 cubic centimeters.

[4] B=the zinc-lime component: $ZnSO_4.6H_2O$, 0.75 gram, plus calcium oxide, 0.5 gram, per 100 cubic centimeters.

When the copper-lime or copper-lime-arsenic mixtures were used the reaction was favorable for growth of all the test organisms, except when the amount required to kill approached 100 percent of the reference formulas. For example, the copper-sulphate-lime-monocalcium arsenite preparation killed all the test fungi at or below 20 percent of its reference formula (table 3), and at the 20-percent concentration had an initial pH value of approximately 8.1. However, mixtures containing some of the less toxic arsenites did not kill until the concentration approached 100 percent of the reference formulae. At the reference concentrations the media had reactions in the range of pH 9 to 10.

RELATION OF THE TOXICITY OF CERTAIN COPPER-LIME-ARSENITE MIXTURES TO THE SUMMATION OF THE TOXICITIES OF THEIR SEPARATE INGREDIENTS

The materials used for making certain copper-lime-arsenite mixtures were studied separately and in the mixtures at reference concentrations suitable for comparing the summation of the indicated toxicities of the separate ingredients of a given preparation with that of the mixture (table 5). Since the copper-lime-monocalcium arsenite mixture was the most toxic material used, killing the test organism, *Physalospora obtusa*, at 5 percent of its reference concentration, its toxicity was given a value of 100 and made the standard from which to compute the relative toxicities of the other materials (5÷lethal concentration of the given material×100).

TABLE 5.—*Increased toxicity of certain copper-lime-arsenite mixtures to Physalospora obtusa as compared with the summation of the toxicities of their separate ingredients*

Formulas or separate ingredients at reference concentrations [1] in grams per 100 cubic centimeters	Concentration [1] required to kill	Relative toxicity [2]	Formulas or separate ingredients at reference concentrations [1] in grams per 100 cubic centimeters	Concentration [1] required to kill	Relative toxicity [2]
	Percent			*Percent*	
Copper sulphate, 0.75	80	6.2	Iron arsenite, 0.5	100	5.0
Calcium oxide, 0.5	100	5.0	A+iron arsenite, 0.5	20	25.0
A [3]	100	5.0	Zinc arsenite, 1.0	150	3.3
Monocalcium arsenite, 0.5	8	62.5	A+zinc arsenite, 1.0	40	12.4
A+monocalcium arsenite, 0.5	5	100.0	Paris green, 1.0	100	5.0
Copper arsenite, 0.5	100	5.0	A+Paris green, 1.0	10	50.0
A+copper arsenite, 0.5	20	25.0			

[1] The formulas or separate ingredients are listed at reference concentrations, such as were used in spraying experiments, and the critical concentrations shown are expressed in percentages of these reference formulas.
[2] Relative toxicity=5÷killing concentration × 100 (see text).
[3] A=the copper-lime component: $CuSO_4.5H_2O$, 0.75 gram, plus calcium oxide, 0.5, per 100 cubic centimeters.

The results presented in table 5 show that each of the copper-lime-arsenite mixtures tested in this experiment, except the one containing zinc arsenite, had a greater toxicity than would be indicated by adding the toxic values of the ingredients that entered into its composition. Copper sulphate, calcium oxide, and monocalcium arsenite, used separately, had relative toxicities of 6.2, 5, and 62.5, respectively, a total of 73.7, as compared with a value of 100 when these same materials were mixed. The zinc, copper, and iron arsenite mixtures showed similar increases in toxicity over the summation of the toxicities of the separate ingredients. Figure 2 shows the very meager growth

made by *Physalospora obtusa* when the copper-lime component and the zinc arsenite were mixed (*A*), as compared with a much increased growth when they were used separately (*B*, *C*). Paris green, used alone, had a relative toxicity of 5, which added to 6.2 for the copper sulphate and 5 for the lime makes a total of 16.2, against a value of 50 when the materials were mixed. If the relative toxicity of the mixture of the copper sulphate and lime, 5, is taken instead of the

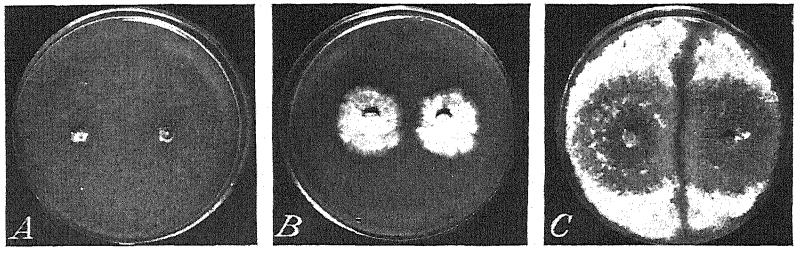


FIGURE 2.—Comparative toxicity to *Physalospora obtusa* of a copper-lime-arsenite mixture and its copper, lime, and arsenical ingredients, respectively: *A*, Copper sulphate 0.015 percent plus lime 0.01 percent plus zinc arsenite 0.01 percent; *B*, copper sulphate 0.015 percent plus lime 0.01 percent; *C*, zinc arsenite 0.01 percent.

relative toxicities of its separate ingredients, 6.2 and 5, the increased toxicity of the copper-lime-arsenite mixtures over the summation of the toxicities of their ingredients is still more notable.

The amount of increased toxicity of the copper-lime-arsenite mixture over that of its separate ingredients varies greatly with the test

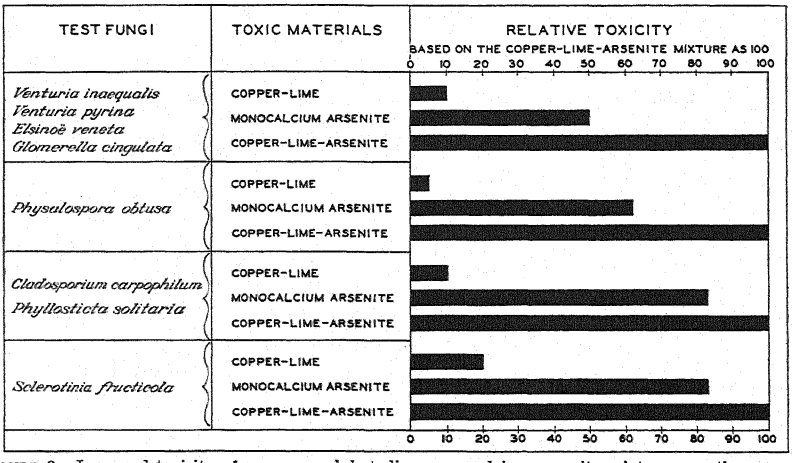


FIGURE 3.—Increased toxicity of a copper sulphate-lime-monocalcium arsenite mixture over the summation of the toxicities of its copper, lime, and arsenical ingredients. The data that apply to more than one test organism are averaged. The composition of the copper-lime-arsenite mixture and the method of computing the relative toxicities are shown in table 5.

fungi, as shown in figure 3. *Physalospora obtusa*, the test organism in the experiment discussed in the preceding paragraph, being quite sensitive, is killed by relatively small amounts of calcium arsenite. Even so, it showed an increased toxicity of the mixture over the summation of the toxicities of the ingredients, amounting to more than 50 percent. With *Venturia inaequalis*, *V. pyrina*, *Elsinoë veneta*, and

Glomerella cingulata, which can tolerate higher concentrations of the separate ingredients, the copper-lime-arsenite mixture had a toxicity 66 percent greater than the added toxicities of the copper, lime, and the monocalcium arsenite ingredients. With *Cladosporium carpophilum* and *Phyllosticta solitaria* only a slight increase in toxicity of the mixture over the added toxicities of its ingredients was indicated; with *Sclerotinia fructicola*, none. In the last case the sum of the indicated toxic values of the separate ingredients was 103. However, this was because the lethal concentration of the copper-lime component was not determined (table 3), the 100-percent concentration being treated in the computation as if it were lethal. Since only *Venturia inaequalis* and *Phyllosticta solitaria* were actually killed by the copper-lime component, the increased toxicity of the mixture to the other test fungi is really greater than that shown in figure 3.

COMPARATIVE EFFECTIVENESS OF COPPER-LIME-ARSENIC MIXTURES AND OF THEIR SEPARATE INGREDIENTS IN SUPPRESSING FUNGAL DEVELOPMENT IN THE VICINITY OF THEIR SOLID RESIDUES

Results of the foregoing experiments and work reported elsewhere (*5*) indicate that copper-lime-arsenic preparations contain or liberate soluble materials capable of exercising fungicidal action at greater or less distance from their solid residues. The following limited studies were undertaken to gain some further evidence regarding the comparative effectiveness of certain copper-lime-arsenic mixtures and of their separate ingredients in suppressing the development of a test fungus in the surrounding medium by means of diffused toxic materials.

The medium used was made of agar, 17 grams; Trommer's malt extract, 5 grams; and water, 1,000 cubic centimeters; adjusted to pH 7. The toxic materials tested were chosen from dusts prepared for seed-treatment experiments, which will be reported elsewhere. One part by weight of each dust material was mixed with 3 parts of the agar medium, and the resulting suspension was allowed to solidify into a slab of standard thickness after thorough agitation to facilitate uniformity of distribution of the test substance. Disks of the toxic agar suspension 5 millimeters in diameter were placed in Petri plates containing uniform amounts of the malt agar, which had been seeded by a standardized procedure with conidia of the test fungus, *Gibberella saubinetii* (Mont.) Sacc. The disks were inserted in the number and positions shown in figure 4, just before the agar solidified. In control plates no toxic disks were inserted. After incubation for 4 days at 20° C., the plates showed a uniform development of the fungus, except in areas about the disks, where it had been killed or inhibited by the diffused toxic materials. The size of the clear areas about the disks is, therefore, an approximate index of the comparative efficiency of the test materials under the experimental conditions. Illustrative results are shown in figure 4. The fungus grew over the entire surface of the control plates (*A*). It grew up to the edge of the copper-lime disks, though it was distinctly inhibited in a narrow zone (*B*). Zinc arsenite was lethal through a comparatively narrow zone (*C*), while monocalcium arsenite was lethal through a zone about twice as wide (*D*). The copper-lime-arsenic preparations were effective through a substantially wider zone than were the arsenical materials that, respectively, entered into their composition (*E*, *F*). Attention

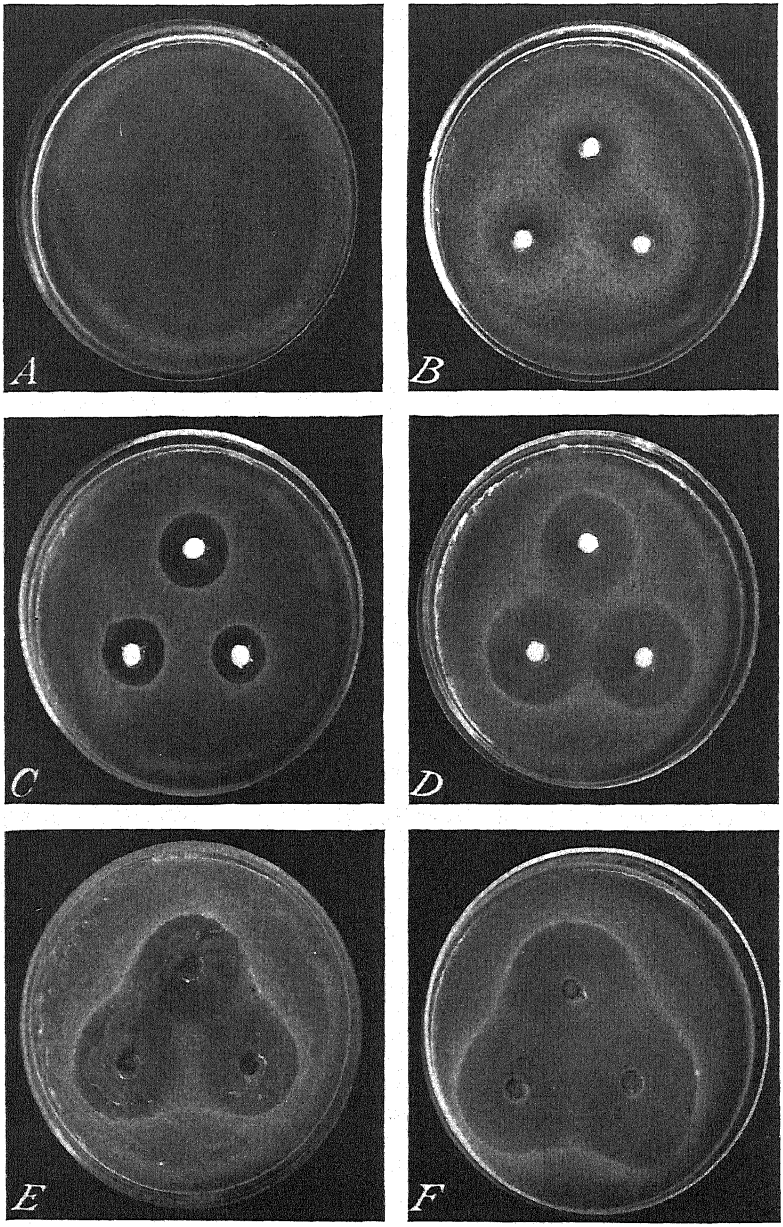


FIGURE 4.—Comparative effectiveness of certain copper-lime-arsenic mixtures and of their separate ingredients in suppressing the development of *Gibberella saubinetii* in a malt-agar medium by means of diffused toxic materials: *A*, Control; *B*, copper-lime mixture (copper sulphate, 1 percent, lime, 1/6 percent); *C*, zinc arsenite; *D*, monocalcium arsenite; *E*, copper-lime-arsenic mixture (copper sulphate, 1 percent, lime, 1/6 percent, monocalcium arsenite, 1/2 percent); *F*, copper-lime-arsenic mixture (copper sulphate, 1 percent, lime, 1/4 percent, monocalcium arsenite, 1/2 percent). The mixtures were prepared as for spraying, filtered, dried, and ground. (See text.)

is invited to the fact that in these experiments the several test materials were compared in equal amounts by weight, rather than in the proportions in which they were used in the mixtures.

In similar experiments the initial reaction of the medium was varied. The copper-lime-arsenic preparations used were capable of exerting a substantial toxic action by means of materials that diffused from the solid residues into the surrounding medium, whether its initial reaction was alkaline, neutral, or acid. Similar toxic action by the copper-lime ingredient was much less at all reactions tried, being practically nil in the alkaline range, slight at neutrality, and somewhat increased in the acid range.

The properties just discussed account in part for the high eradicant effectiveness of copper-lime-arsenic mixtures against fungal fruiting structures, and suggest possible adaptations of these materials for combating seed- or soil-borne pathogens.

DISCUSSION

The literature on the toxicity and fungicidal use of arsenical compounds and copper-lime-arsenic mixtures has been surveyed by Keitt and Palmiter (*5*).

In confirmation of the conclusions of Keitt and Palmiter (*5*), the results of the toximetric experiments reported herein show that suitable copper-lime-arsenic mixtures have powerful fungicidal properties, and that their toxicity can be varied through a wide range by modifications of formula. A striking feature of these studies is the high and comparatively uniform toxicity of certain copper-lime-arsenic mixtures to all the test fungi used.

The fact that the toxicity of the copper-lime-arsenite mixtures studied was usually greater than the summation of the toxicities of their separate ingredients is of interest, both in relation to their superior fungicidal effectiveness in spraying experiments (*5*) and for theoretical considerations. The reasons for this increased toxicity of the mixtures have not been investigated. It is noteworthy, however, that these mixtures contain both a toxic anion and a toxic cation. Furthermore, there are manifold possibilities of changes in toxicity incident to chemical reactions and physiochemical changes in such complex preparations.

The ability of suitable copper-lime-arsenic mixtures to liberate soluble materials capable of diffusing through an acid, neutral, or alkaline medium and exercising fungicidal action at considerable distances from the undissolved residues is an important factor in their effectiveness as fungicides (*5*).

SUMMARY

The toxicity of certain copper-lime-arsenic mixtures and of their separate ingredients was studied by a modification of the method of Schmitz et al., using as test organisms *Venturia inaequalis*, *V. pyrina*, *Cladosporium carpophilum*, *Phyllosticta solitaria*, *Elsinoë veneta*, *Glomerella cingulata*, *Physalospora obtusa*, and *Sclerotinia fructicola*.

Suitable copper-lime-arsenic mixtures were highly toxic to all the test fungi. The toxicity of such mixtures could be varied through a wide range by modifications of formula.

Copper sulphate-lime mixtures had relatively low toxicity.

The toxicity of the arsenical compounds tested varied through a wide range. Monocalcium arsenite was the most toxic, usually being from 3 to more than 10 times as toxic as copper sulphate, depending on the test fungus used. Tricalcium arsenite was slightly less toxic. Zinc and iron arsenites were comparatively low in toxicity, while paris green and copper arsenite were intermediate. Tricalcium arsenate was the least toxic arsenical compound tested, and dicalcium arsenate was only slightly more toxic.

Six monoconidial isolates of *Venturia inaequalis* showed about as much variation in reaction to copper sulphate and certain arsenites, respectively, as did species of six other genera.

The toxicity of the copper-lime-arsenite mixtures studied usually exceeded the summation of the toxicities of their separate ingredients.

It is shown in tests with *Gibberella saubinetii* that suitable copper-lime-arsenic mixtures liberate soluble materials capable of diffusing through an acid, neutral, or alkaline medium and exercising fungicidal action at considerable distances from the undissolved residues.

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RESTORATION OF VIRULENCE OF ATTENUATED CURLY TOP VIRUS BY PASSAGE THROUGH SUSCEPTIBLE PLANTS [1]

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INTRODUCTION

The sugar-beet curly top virus, after passage through *Chenopodium murale* L., was shown by Carsner [3] to produce mild effects when subsequently inoculated into sugar-beet plants. This indicated change enough to warrant the designation "attenuated virus." The writer has shown that other plants may function similarly [4] and that virulent curly top virus, after it has been attenuated by passage through *C. murale*, may be restored to its original condition of virulence, or nearly so, by a single passage through chickweed, *Stellaria media* (L.) Cyr.[5]

In nature, restoration of virulence by passage through *Stellaria media* probably does not play an economic role, since this plant is common only in moist, shady locations, unfavorable to the curly top vector, *Eutettix tenellus* (Baker). If, however, sugar beets in a very susceptible stage or some of the important weed hosts of the beet leafhopper known to be susceptible to curly top were found to act in a manner similar to that of *S. media* in restoration of virulence to attenuated virus, the findings might have a bearing on the curly top situation. Work was undertaken on sugar beets in the seedling stage and on alfileria, *Erodium cicutarium* (L.) L'Hér., a host plant occurring in considerable abundance in many leafhopper breeding areas. Later, peppergrass, *Lepidium nitidum* Nutt., which is also an important host, was tested. These plants were known to be highly susceptible to virulent curly top virus. In the case of alfileria, it is known that the curly top virus can be carried through the winter on this species and it may be a winter and early-spring host plant in California for the beet leafhopper. Severin [6] includes peppergrass as one of the important overwintering annuals for this insect.

MATERIALS AND METHODS

The experimental procedure used in the tests has been described.[7] The virulent curly top virus was first attenuated by passage through *Chenopodium murale*. This attenuated virus was then inoculated by means of leafhoppers into the susceptible plant, after which the viru-

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[2] Acknowledgment is made to Eubanks Carsner and N. J. Giddings, senior pathologists, and G. H. Coons, principal pathologist, Division of Sugar Plant Investigations, for their criticisms in the preparation of this manuscript.

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lence of the virus was determined by transmission to young, fast-growing sugar beets in the two- to four-leaf stage. As a control in each test, inoculations were made with the virulent virus before and after its passage through *C. murale*.

In the first experiments with alfileria, the type of symptoms was described as mild or severe as in the work reported for *Stellaria media*.[7]

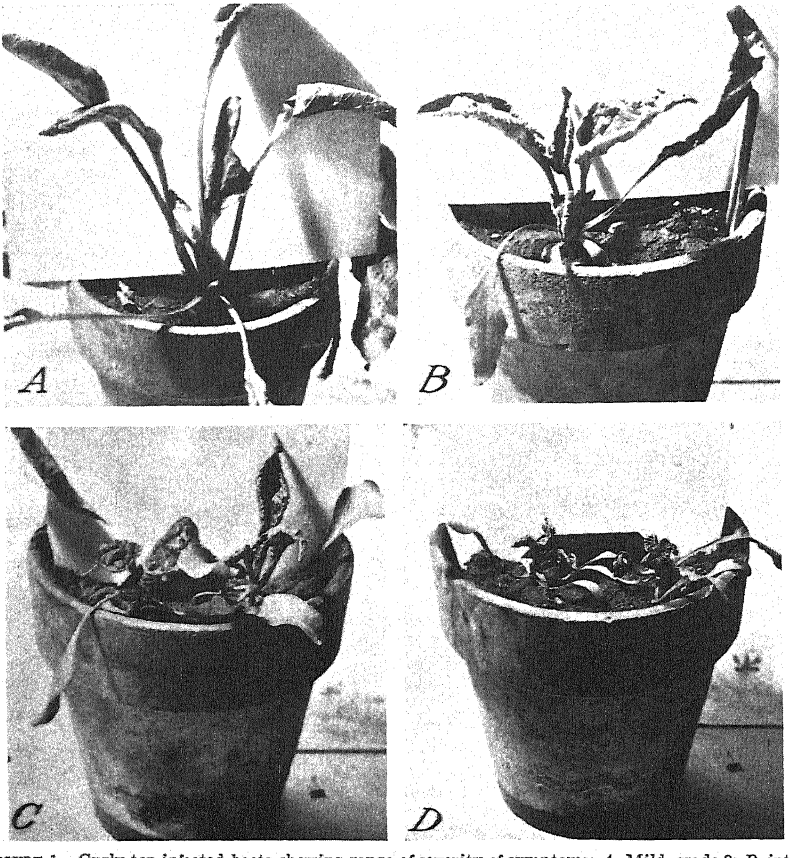


FIGURE 1.—Curly top-infected beets showing range of severity of symptoms: *A*, Mild, grade 2; *B*, intermediate, grade 3; *C*, more severe, grade 4; and *D*, extreme dwarfing, grade 5. (Grade 1, mild response characterized by veinlet clearing only, not illustrated.)

In later experiments a grading system to express severity of the symptoms on test plants as developed by Giddings [8] was adopted. The affected plants were classified in five grades based on response, as follows:

(1) *Mild response.*—No stunting or dwarfing; veinlet clearing or slight vein enlargement only visible symptoms.

(2) *Mild response.*—Little or no dwarfing; veinlets cleared, vein roughening and vein protuberances on some leaves, and leaves sometimes curled (fig. 1, *A*).

(3) *Intermediate response.*—Some dwarfing and stunting, practically all leaves involved; leaves somewhat curled, vein roughening on nearly all leaves (fig. 1, *B*).

[7] LACKEY, C. F. See footnote 5.

[8] GIDDINGS, N. J. A GREENHOUSE METHOD FOR TESTING RESISTANCE TO CURLY TOP IN SUGAR BEETS. Phytopathology 27: 773-779, illus. 1937.

(4) *More severe reaction.*—Dwarfing severe, leaves markedly curly or distorted, veins conspicuously distorted and roughened (fig. 1, *C*).

(5) *Extreme dwarfing.*—All symptoms of preceding grade very pronounced; plants nearly killed (fig. 1, *D*).

EXPERIMENTAL TESTS WITH SUGAR BEETS

Sugar beets (*Beta vulgaris* L.) in the cotyledon stage, the two- to four-leaf stage, and the six- to eight-leaf stage were tested for their effect on the attenuated virus from *Chenopodium murale*. The cotyledon beets showed 63.8 percent infection with an average severity of

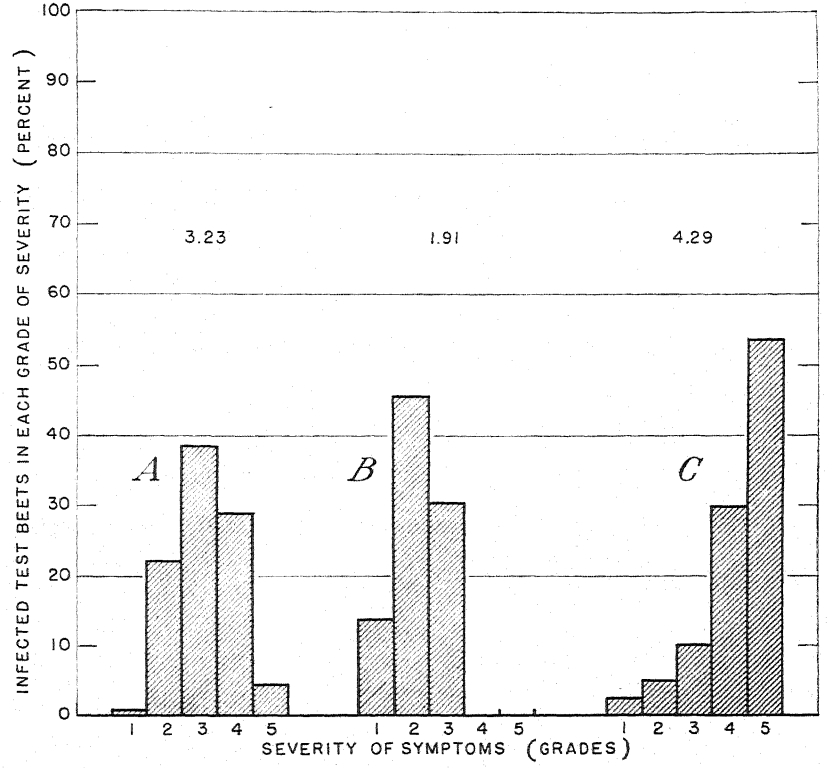


FIGURE 2.—Histograms showing distribution of symptoms in grades of severity in test beets infected with attenuated curly top virus before and after passage through cotyledon-size sugar beets: *A*, Attenuated virus after passage through cotyledon beets; *B*, attenuated virus untreated; *C*, original virulent virus. (Numbers above histograms indicate average grade of severity of symptoms for each treatment.)

2.31; two- to four-leaf beets, 42.2 percent infection with an average severity of 1.91; and the six- to eight-leaf beets, 34.0 percent infection with an average severity of 1.34. Twenty-three tests in which two- to four-leaf beets were inoculated with the original virulent virus gave an average grade of severity of 4.29 (fig. 2). Table 1 is a summary of the results of 2 years' trials with these various-sized beets.

The cotyledon beets showing symptoms severe enough to grade 4 and 5, comprising 13.4 of those infected, were tested for their effect on the attenuated virus. It is evident (table 2) that some degree of restoration was obtained by passage through cotyledon beets. In the course of these tests with sugar beets, two cases occurred in which

some increase of virulence resulted from passage of the virus through sugar beets in the two-leaf stage. Beets with six to eight leaves did not increase the virulence of the attenuated virus. Table 2 summarizes the results of these experiments, which are presented in more detail in the histograms in figure 2.

As an additional measure of the effect of passing the attenuated virus through cotyledon beets, the infected test beets in several experiments were weighed 8 to 10 weeks after inoculation. The beets inoculated with the attenuated virus averaged 15.16 g, green weight; while those inoculated with the restored virus averaged 7.87 g.

TABLE 1.—*Summary of all experiments in which sugar beets in the cotyledon stage and those in the 2- to 8-leaf stages were inoculated with the attenuated virus*

Size of beets when inoculated	Beets inoculated	Beets infected	Average grade of severity	Infected beets in each grade of severity [1]				
				1	2	3	4	5
	Number	*Percent*		*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
Cotyledon stage	304	63.8	2.31	18.0	46.9	21.1	10.8	2.6
2- to 4-leaf stage	95	42.2	1.91	38.0	40.5	4.7	9.5	0
6- to 8-leaf stage	103	34.0	1.34	68.6	31.4	2.9	0	0

[1] Percentages based on number of infected beets.

TABLE 2.—*Restoration of virulence of attenuated virus by passage through cotyledon beets*

Treatment of virus used	Experiments	Test plants inoculated	Test plants infected	Average grade of severity
	Number	*Number*	*Percent*	
Passed through cotyledon beets	32	500	73	3.23±0.11
Not passed through cotyledon beets	25	235	59	1.91± .10

EXPERIMENTAL TESTS WITH ERODIUM CICUTARIUM (ALFILERIA)

Evidence from the first experiments indicating that passage of the attenuated virus through alfileria may restore the virulence is shown in table 3. All cases in which some degree of restoration was obtained are included in this résumé. From the data given in table 3, it is clear that after a single passage attenuated virus may be so restored in virulence as to produce severe symptoms on the test beets. These results are comparable to those reported for *Stellaria media* [9] in that there were many cases in which alfileria did not restore the virulence of the attenuated virus.

TABLE 3.—*Results of passing the attenuated and virulent forms of curly top virus through alfileria*

Treatment and form of virus used	Beets inoculated [1]	Beets infected	Type of symptoms
Not passed through alfileria:	*Number*	*Percent*	
Virulent	5	60	Severe.
Attenuated	25	8	Very mild.
Passed through alfileria:			
Virulent	40	60	Severe.
Attenuated	52	52	Do.

[1] 3 to 6 leafhoppers were fed on each test beet for 3 to 4 days.

[9] LACKEY, C. F. See footnote 5.

The results of later and more extensive experiments are summarized in table 4. Only 50 percent of the alfileria plants inoculated with attenuated virus showed symptoms of curly top. About 90 percent of these diseased plants were tested for their effect on the attenuated virus. Of these, only 20 percent increased the virulence of the virus to some degree. Before passage through alfileria, the attenuated virus produced only 32 percent infection on the test beets, with an average grade of severity of 1.82, as compared with 63 percent infection, with an average grade of 3.23, after passage through alfileria. This demonstrates that alfileria occasionally increases the virulence of the attenuated virus.

TABLE 4.—*Restoration of virulence of attenuated virus by passage through alfileria*

[Summary of experiments conducted at Riverside, Calif., 1931–34]

Treatment of virus used	Experiments	Test plants inoculated	Test plants infected	Average grade of severity
	Number	*Number*	*Percent*	
After passage through alfileria	55	1,574	63	3.23±0.09
Before passage through alfileria	45	840	32	1.82± .06

In 17 of these experiments test beets were inoculated with the original virulent virus. The average grade of severity of symptoms produced by this virus was 4.22 (fig. 3).

The histograms in figure 3 show what percentage of the infected test beets fell into each grade of severity. The test beets infected with the attenuated virus after passage through alfileria showed symptoms that placed a majority of them in grades 3 and 4 and a few in grade 5. Figures 4 and 5 show this variation in degree of severity of symptoms expressed by the attenuated virus after restoration. On the other hand, the attenuated virus before treatment produced symptoms which in the greatest number of cases graded 1 and 2. While most of the test beets inoculated with the original virulent virus showed symptoms grading 4 and 5, some of these plants exhibited symptoms mild enough to put them in grade 1, 2, or 3. As shown in studies on restoration through *Stellaria media*,[10] the appearance of mild symptoms on some of the test beets that had been inoculated with the restored virus or with the original virulent virus appears to be an individual plant response in some instances. An apparently comparable situation sometimes develops in the case of alfileria, as illustrated in figure 5. Transferring virus from such mildly affected plants to test beets resulted in severe symptoms in the majority of cases.

EXPERIMENTAL TESTS WITH LEPIDIUM NITIDUM (PEPPERGRASS)

The attenuated virus used in the test with *Lepidium nitidum* was from two sources. The first lot had been attenuated by passage through *Chenopodium murale*, as in all the previously mentioned experiments on restoration. The second lot was obtained from leafhoppers collected in the field from natural breeding areas. Only 40 percent of the *L. nitidum* plants inoculated with the attenuated virus became diseased, and 70 percent of these infected plants increased the virulence of the virus. The histograms in figure 6 show the results of these tests. The attenuated virus harbored by field collections

[10] LACKEY, C. F. See footnote 5.

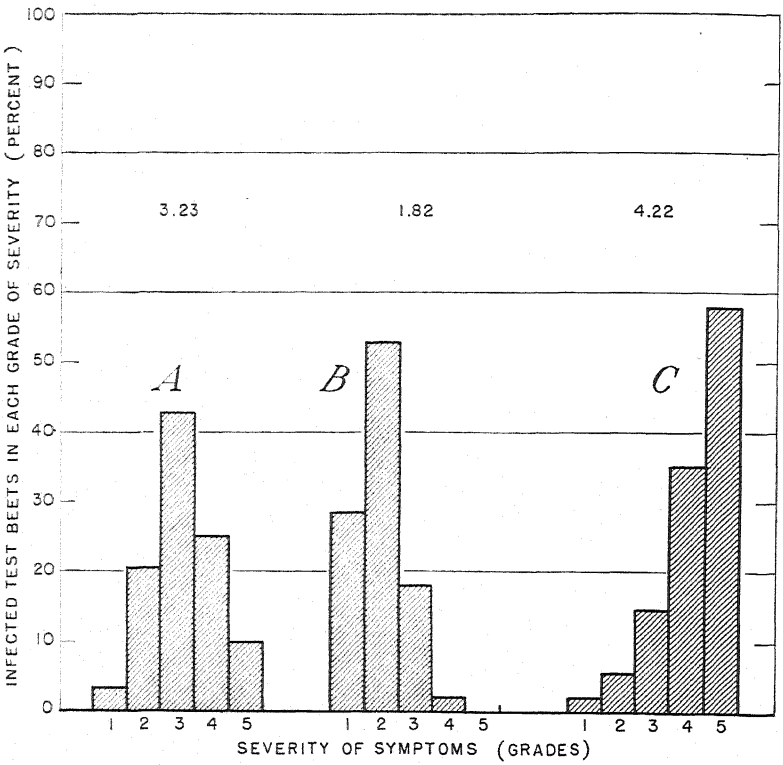


FIGURE 3.—Histograms showing distribution of symptoms in the different grades of severity on test beets infected with the attenuated virus before and after passage through alfileria: *A*, Attenuated virus after passage through alfileria; *B*, attenuated virus untreated; *C*, original virulent virus. (Numbers above histograms indicate the average grade of all test beets for the different treatments.)

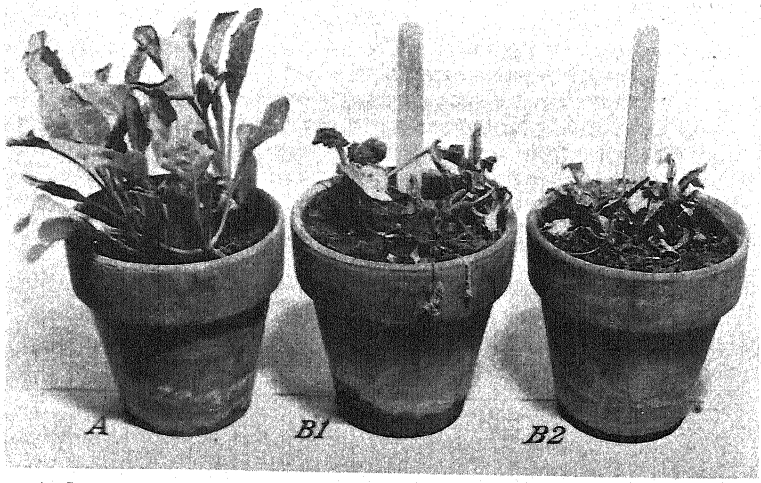


FIGURE 4.—Symptoms produced on test beets by restored and by untreated attenuated virus: Pot *A*, by attenuated virus before passage through alfileria; pots *B*1 and *B*2, by the same virus after passage through alfileria.

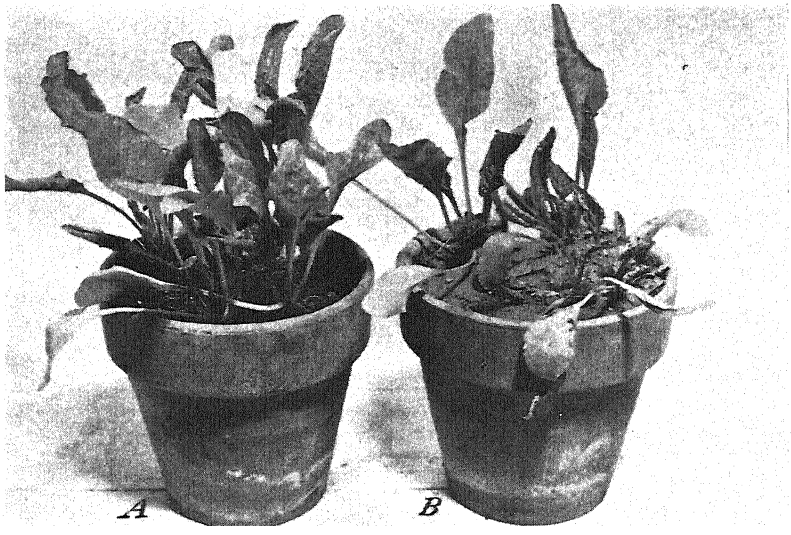


FIGURE 5.—Symptoms produced by the restored and the untreated attenuated virus on test beets: Pot *A*, by attenuated virus before passage through alfileria; pot *B*, by the same virus after passage through alfileria, showing variation in individual plant response to infection.

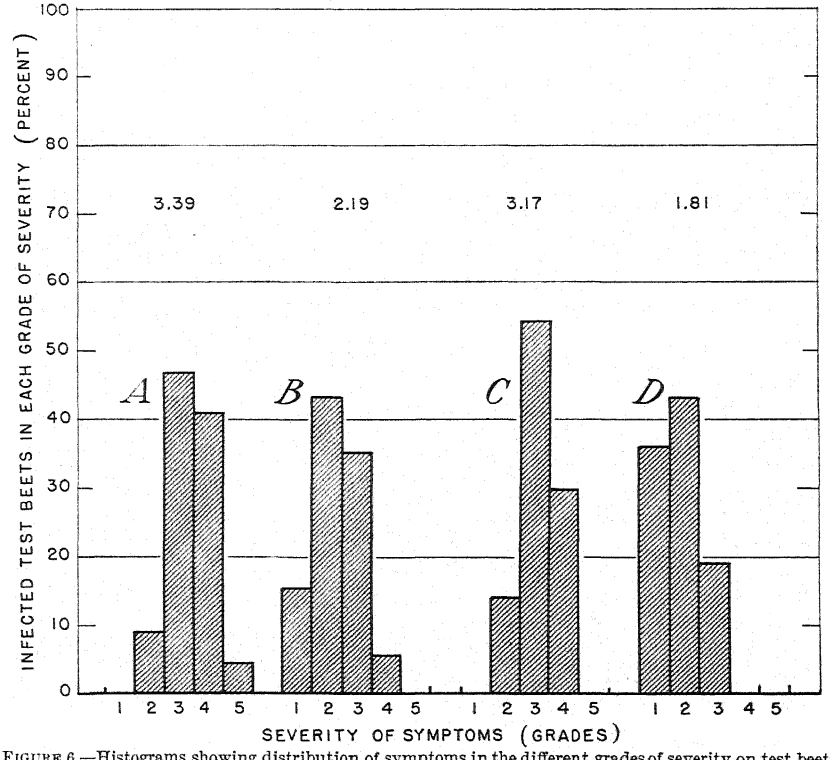


FIGURE 6.—Histograms showing distribution of symptoms in the different grades of severity on test beets infected with the attenuated virus before and after passage through *Lepidium nitidum*: *A*, Attenuated virus from field collection of insects, passed through *Lepidium nitidum*; *B*, attenuated virus, from field collection, untreated; *C*, attenuated virus, from *Chenopodium murale*, passed through *Lepidium nitidum*; *D*, attenuated virus, from *C. murale*, untreated. (Numbers above histograms indicate average grades of severity.)

of leafhoppers, with an average grade of 2.19, was not attenuated so much as the virus that passed through *C. murale*, with an average grade of 1.81. It would appear at first glance at the histograms that the attenuated virus from the field leafhoppers was restored to a greater degree, but it showed an increase in the average grade of severity of only 1.20 grade points while the attenuated virus from *C. murale* showed an increase of 1.36 grade points. The reaction to passage through peppergrass by the attenuated virus from the two sources is therefore very similar. Table 5 summarizes all the tests made with these two lots of attenuated virus. The average grade of severity before and after treatment is practically the same as that in the restoration tests with alfileria.

TABLE 5.—*Restoration of virulence of attenuated virus by passage through Lepidium nitidum*

Treatment of virus used	Experiments	Test plants inoculated	Test plants infected	Average grade of severity
	Number	*Number*	*Percent*	
After passage through *Lepidium nitidum*	13	135	84.51	3.26±0.27
Before passage through *Lepidium nitidum*	11	85	69.41	2.10± .50

DISCUSSION

The economic bearing of the studies with wild hosts on the curly top problem will require further investigation in the field. However, the experimental evidence with young sugar beets suggests that an increase in the virulence of the virus may occur under field conditions also.

Observations on alfileria indicated that virulence restoration occurred most frequently in plants that were growing rapidly. To test the correctness of this idea, plants of different ages were fertilized with nitrogen (ammonium sulphate) 10 days before inoculation. When very young alfileria plants or those given extra nitrogen were used, a greater number became severely diseased and restored the virus. Similarly, inoculation with eight leafhoppers resulted in more cases of severe infection and consequently more plants producing restoration than when inoculations were made with two leafhoppers. None of these various procedures had any pronounced effect on the degree of restoration attained.

SUMMARY

Virulent curly top virus, attenuated by passage through *Chenopodium murale*, has been occasionally restored in virulence by passage through cotyledon-sized sugar beets.

The appearance of mild symptoms on a few test beets inoculated with restored virus or with the original virulent virus seems in some cases to be an individual plant response, since virus from some of these beets produces severe symptoms on the majority of young test beets to which it is transferred.

Alfileria (*Erodium cicutarium*) and peppergrass (*Lepidium nitidum*) also occasionally restore the virulence of the attenuated curly top virus. These plants are important overwintering hosts for both the leafhopper vector and the virus.

The probable economic aspect of these investigations is suggested.

BIOLOGICAL VALUE OF CASEIN AS A SUPPLEMENT TO THE PROTEINS OF BARLEY IN RATIONS FOR PIGS[1]

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Animal husbandman, California Agricultural Experiment Station

INTRODUCTION

It has been shown at the California Agricultural Experiment Station by Thompson and Voorhies (*7*)[2] and by Hughes (*2*) that barley, when fed as the only source of protein, does not produce optimum growth in pigs. Protein-rich concentrates like fish meal and tankage or dairy byproducts when used as supplements to barley, produce more rapid growth. Such concentrates, however, contain other nutrients than protein, which makes it unsafe to conclude that the protein is responsible for the results obtained. Casein, since it has been used by biologists in similar studies with smaller animals than the pig, and since it is relatively low in cost, was used as a supplement in the studies reported here to determine whether or not the proteins of barley are present in amounts necessary for the rapid development of the pig.

EXPERIMENTAL DATA

In June 1929 two pigs about 50 pounds in weight, having free access to direct sunlight, were put on a diet of rolled barley, salt, and cod-liver oil. They grew very slowly and became stiff in the joints. Early in January of the following year calcium carbonate was added to the diet. At 1 year of age the pigs weighed only 162 pounds. It was believed that the diet was sufficient in all known factors for some growth, but the pigs had not gained in weight for some time. The only protein in the ration was that of barley. Casein was added to the diet and an immediate response was manifested by increase in weight, thrift, and appetite. During the following 3 months the pigs made an average daily gain of slightly more than 1 pound and became normal in health.

The results of this experiment were significant, but the number of animals used was too small to make possible any definite conclusions; therefore, a similar experiment on a larger scale was conducted in 1931–32. Two groups of eight pigs each were divided as nearly as possible into comparable lots. Each group was placed in a concrete lot with free access to direct sunlight, and the pigs were fed all they would consume of the following diets:

Group 1. Rolled barley, 98 percent; sodium chloride, 1 percent; calcium carbonate, 1 percent; and 5 cc of cod-liver oil each daily (basal diet).

Group 2. Rolled barley, 96.5 percent; sodium chloride, 1 percent; calcium carbonate, 1 percent; casein, 1.5 percent; and 5 cc of cod-liver oil each daily.

The results of this experiment are summarized in table 1.

[1] Received for publication Mar. 9, 1937; issued October 1937.

[2] Reference is made by number (italic) to Literature Cited, p. 465.

The average beginning and final weights were, for group 1, 23.3 and 94.6 pounds; and for group 2, 25.0 and 203.8 pounds. During the first part of the experiment the pigs fed casein (group 2) gained three times as fast and were more economical in their use of food than were the pigs fed protein supplied only by the barley (group 1). Each pound of casein fed to the animals in group 2 had a replacement value of 49.5 pounds of the basal feed. When casein was added to the diet of group 1, at both the 1.5- and 5-percent levels, there was an immediate response in increased appetite, rate of gain, and economy of feed utilization. The pigs did not gain as rapidly at either level as the pigs in group 2; however, they did consume less food for a unit of increase. It is postulated that this was due to a lower maintenance requirement because of a marked difference in the size of the pigs in both lots. When the casein level was increased to 5 percent the animals in both groups gained more rapidly and consumed less food for 100 pounds of gain than at any other time during the progress of the experiment, the protein level being apparently more nearly optimum for pigs at this stage of growth and development. The rate of gain in both groups was just about twice as rapid as when casein was fed at the 1.5-percent level.

TABLE 1.—*Summary of data from the second and third experiments to show the effect of adding casein to the basal diet of pigs when the sole other source of protein was barley*

Feeding period and diet	Group 1		Group 2	
	Average daily gain	Feed consumed per 100 pounds gain in weight	Average daily gain	Feed consumed per 100 pounds gain in weight
SECOND EXPERIMENT [1]				
	Pounds	*Pounds*	*Pounds*	*Pounds*
May 23 to Dec. 12, 1931 (group 1, basal diet; group 2, 1.5 percent casein added)	0.16	886.7	0.50	508.9
Dec. 12, 1931, to Jan. 23, 1932 (both groups, basal diet plus 1.5 percent casein)	.39	493.9	.77	529.9
Jan. 23 to Feb. 13, 1932 (both groups, basal diet plus 5 percent casein)	.88	310.0	1.45	339.3
Feb. 13 to Mar. 5, 1932 (both groups fed basal diet only)	.14	2,265.0	.74	711.3
THIRD EXPERIMENT [2]				
May 12 to Oct. 6, 1934 (group 1, basal diet; group 2, 1.5 percent casein added)	.36	577.6	.62	431.8
Oct. 6, 1934, to Jan. 10, 1935 (both groups, basal diet plus 1.5 percent casein)	1.20	[3] 445.1	1.37	438.9

[1] Groups of 8 pigs each.
[2] Groups of 10 pigs each.
[3] One pig was "off feed" for some time and died the day the experiment closed.

While the last period was only a short one, the results are significant because of the immediate decrease in daily gain by both groups and the large increase in food consumption required for a unit of increase in weight. The pigs in group 1 reverted to their original rate of gain, and while the pigs in group 2 were much larger and were fattening, their rate of increase dropped from 1.45 to 0.74 pounds per head daily.

A third experiment was conducted in 1934 to further check the results already obtained. This experiment was begun May 12, 1934,

and concluded January 10, 1935. Two groups of 10 pigs each were fed diets similar to those previously used. The average beginning weight for both groups was 29.6 pounds. The conditions of the experiment, including the grouping of the pigs and the size of the pens, were the same as before. To be sure that a lack of vitamin A would not influence the results, each pig in both groups was given 5 cc of cod-liver oil daily until July 21, and from that time until the experiment was concluded each was given 10 cc. The results are presented in table 1.

In this experiment the pigs were larger and thriftier at the beginning than those of the second experiment and were not fed so long in the first period, which may in part account for the difference in the rate of gain and the replacement value of the protein when the results are compared with those of the second experiment. Each pound of casein fed group 2 had a replacement value of 22.5 pounds in terms of food saved over that fed group 1. During the second phase of this experiment the pigs in both groups gained more rapidly than in the first period, which is in agreement with the results of the other experiments. While in some details the results of this test differed slightly from those of the preceding one, in principle they were the same.

In 1936 a fourth experiment was concluded which differed somewhat from those already reported. Osborne and Mendel (*3*) had reasoned that the failure of cereal proteins as they are generally fed is due to a lack of sufficient total cereal protein. In an effort to prove or disprove this theory and to obtain further information on the original subject, barley proteins fed group 1 were increased to a point in excess of that fed group 2, which received barley and casein. This was done by adding dried brewers' grains from which some of the hulls had been removed (the material removed was a mixture of hulls and some grain). Since the addition of dried brewers' grains to the diet of group 1 increased the fiber content, barley hulls were added to the mixture fed group 2 to make it comparable in this respect. The total crude protein fed groups 1 and 2 was respectively 11.12 and 10.42 percent. The fiber content of the ration fed to group 1 was 1 percent higher than that of the ration fed group 2. It is probable, however, that there was little difference in the amount of fiber consumed by the pigs in the two groups since those of group 1 left considerable quantities of hulls in the bottom of their feeder.

Since commercial casein contains lactoflavin, and the results of other experiments with hogs at this station (unpublished data had indicated that this factor is necessary for normal growth, it was deemed expedient to feed a third group of pigs casein washed free of this factor. The casein was washed by the method of Evans, Lepkovsky, and Murphy (*1*), modified by that of Supplee, Flanigan, Hanford, and Ansbacher (*6*).

The crude protein and fiber content of the diets fed groups 2 and 3 were the same.

The diets were:

Group 1. Rolled barley, 88 percent; brewers' grains (dried), 10 percent; sodium chloride, 1 percent; calcium carbonate, 1 percent; and 10 cc of cod-liver oil each weekly.

Group 2. Rolled barley, 91 percent; barley hulls, 5 percent; casein, 1.5 percent; sodium chloride, 1 percent; calcium carbonate, 1.5 percent; and 10 cc of cod-liver oil each weekly.

Group 3. The same as for group 2 except that the casein fed was washed free of lactoflavin.

The physical conditions of this experiment, including the grouping of the pigs and the size of the pens, were comparable to those of the other experiments. The experiment was begun June 6 and concluded October 31, 1936. There were eight pigs in groups 1 and 2 and five pigs in group 3.

TABLE 2.—*Summary of data from the fourth experiment to show the effect of adding commercial casein and casein washed free of lactoflavin to the basal diet of pigs when the sole other source of protein was barley*

[Experiment begun June 6 and concluded Oct. 31, 1936; 8 pigs in groups 1 and 2 and 5 in group 3]

Group no.	Average initial weight	Average daily gain	Feed consumed per 100 pounds gain in weight
	Pounds	*Pounds*	*Pounds*
1	39.3	0.61	383.0
2	38.8	1.12	320.4
3	34.4	.57	423.7

In this experiment as in the others the pigs fed casein (group 2) gained much faster and consumed less feed for 100 pounds of gain than those (group 1) fed only barley proteins, even though the total protein in the diet of group 1 was greater than that of group 2. In this case each pound of casein in the diet of group 2 had a replacement value of about 13 pounds of the diet fed group 1. However, if the results of this experiment are compared with those for animals fed only barley as shown in table 1, it will be found that the additional protein supplied by brewers' grain did result in increasing the daily gains and in decreasing the amount of feed required for 100 pounds of gain.

The pigs in group 3 (fed casein washed free of lactoflavin) gained no faster than the pigs of group 1 and consumed more feed for a unit of increase than either of the other groups.

DISCUSSION

In the experiments reported herein where the proteins of barley were the only source of protein for young growing pigs the rate of growth was slow. This agrees with the finding of Steenbock, Kent, and Gross (*5*) and with that of McCollum, Simmonds, and Parsons (*3*). Osborne and Mendel (*4*), however, reported that barley proteins are adequate as a whole in the nutrition of growth, and that several of their rats grew to large adult size without any other source of protein. The growth increase in their rats, however, was not consistent.

The addition of casein to the diet in the present experiments reduced considerably the amount of feed required for a unit increase in weight. Its value in replacing barley varied; however, 1 pound of casein supplanted more than 20 pounds of barley, except in the fourth experiment when it replaced about 13 pounds. In this case barley proteins were increased by the addition of dried brewers' grains to a point above that of the other group fed barley and casein. The results of these studies indicate that barley is deficient in some essential factor or factors necessary for normal growth and weight increase in the young pig.

That the total amount of protein in barley is probably a factor of importance is indicated by the fact that when dried brewers' grains were added to barley, thereby increasing the total barley proteins fed, the gains made were greater and the amounts of feed consumed for a unit of gain were smaller than when barley was fed alone.

Some uncertainty exists as to the reason for the beneficial effect of the addition of casein. The quantity of the protein and the lactoflavin content of the casein have been considered. Pigs fed unwashed commercial casein (group 2, experiment 4) gained twice as fast as pigs fed the same diet at the same time except that casein (from the same sack) washed free of lactoflavin was used.

SUMMARY

Young growing pigs fed a diet in which the proteins of barley were the only source of protein grew very slowly and required large quantities of feed for a unit of increase in weight.

When casein was added to such a diet there resulted a marked increase in rate of growth and a large reduction in feed required for an increase in body substance.

When the barley proteins in the diet were increased by the addition of dried brewers' grains, the gains were more rapid and the feed consumed for 100 pounds of gain were less than when only barley was fed.

The possibility that the lactoflavin in casein is responsible for its beneficial effect when added to barley is suggested.

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EFFECT OF PROTEIN DEFICIENCY IN THE RATION ON THE AMOUNT OF FEED CONSUMED BY LAMBS[1]

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INTRODUCTION

In studies of the nutritive value of proteins in which biological values are to be calculated (*3*),[3] it is necessary to know the losses of endogenous and metabolic nitrogen. To measure such losses, the experimental animals are fed diets which contain no nitrogen, or very little. Since diets of this type are not only deficient in protein, but are radically different from ordinary diets, certain difficulties have been experienced in using them.

A serious limitation in the use of most of the nitrogen-free or low-nitrogen diets has been the difficulty of obtaining adequate feed consumption. With the possible exception of swine, it has been found almost impossible to induce experimental animals to consume satisfactory amounts of these diets for the desired length of time. In most cases when such diets were readily consumed, they contained a certain amount of some high-protein feed which was added specifically to increase the palatability.

A palatable ration, free from nitrogen or very low in nitrogen, has been particularly difficult to devise for lambs. An attempt was made at this station (*7*) to feed lambs a purified diet almost free from nitrogen and containing no natural roughage, but the ration was not sufficiently palatable to the lambs and its use was discontinued. Workers who have used low-nitrogen rations for lambs (*4, 5, 6, 7, 8*) have included straw as part of the ration, but even when this was done the feed intake on the low-nitrogen rations was decidedly smaller than on the experimental rations containing adequate protein, and in practically all cases the lambs lost in weight.

The object of the present study was to devise a low-nitrogen ration, containing only purified ingredients, which would be consumed readily by lambs over a reasonable length of time. Such a ration was desired for use in experiments conducted at the Cornell Station on the nutritive value of proteins.

PRELIMINARY TRIALS

Considerable work has been done at this station in developing purified or synthetic diets for Herbivora (*2, 9*). A diet for sheep which has given good results over a long period of feeding is as follows: Regenerated cellulose,[4] 20 percent; casein, 20 percent; sucrose, 15 percent; starch, 28 percent; yeast, 7 percent; salt mixture,[5] 4 percent;

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[3] Reference is made by number (italic) to Literature Cited, p. 473.

[4] This product was obtained as washed Sylphrap from the Sylvania Industrial Corporation, New York City.

[5] The salt mixture used was one designed by Woodward and McCay (*9*) for use in synthetic diets for Herbivora.

lard, 6 percent; and a vitamin A–D supplement. This diet, as well as other very similar diets, is apparently palatable to sheep and has been consumed in such amounts that good growth has resulted. However, it should be noted that this mixture contains a high percentage of protein.

With this work as a basis, the most logical procedure to follow in developing a low-nitrogen diet was apparently to replace the casein with starch. This change would not be expected to affect the palatability of the mixture very radically. That some difficulty might be encountered, however, became apparent, after some preliminary work which was made in connection with another problem.

In the preliminary study, a growing lamb which previously had been getting a common, practical ration was fed a diet containing 18 percent of casein but otherwise similar to the purified diet described above. No trouble was experienced in getting satisfactory feed consumption, and so it was concluded that this 18-percent casein diet was palatable to the lamb. Then, by using a larger percentage of starch and a smaller percentage of casein, another diet was prepared in which the casein made up only 8 percent of the total ration. When first fed this mixture seemed just as palatable as the 18-percent casein diet and the same amount of feed was consumed. At the end of 6 days, however, the lamb went off feed and was emaciated in appearance. At about the same time the feces became loose in character and offensive in odor, indicating acute indigestion. Although the feeding of this diet was continued for 12 days longer, the lamb made little or no improvement and the former level of feed consumption was not regained.

The lamb was then fed a similar diet except that it contained 14 percent of casein. After a short time the lamb's appetite became stronger and more regular, the feces were more normal, and the lamb was apparently in considerably better physical condition.

From these results it seemed unlikely that omitting all the casein from the diet and adding a corresponding amount of starch would make a satisfactory low-nitrogen diet for lambs. Therefore, certain other modifications were made to the original purified diet in addition to the substitution of starch for casein. This mixture, which was used in all the remaining studies on low-nitrogen diets, is given in table 1. Another diet, similar in all respects except that 20 percent of casein replaced a corresponding amount of starch, was also used. This diet, hereafter referred to as the "casein diet", is also given in table 1.

TABLE 1.—*Composition of experimental diets*

Ingredients	Low-nitrogen diet	Casein diet	Ingredients	Low-nitrogen diet	Casein diet
	Percent	*Percent*		*Percent*	*Percent*
Regenerated cellulose	18.0	18.0	Yeast	5.0	5.0
Casein		20.0	Minerals [1]	4.5	4.5
Starch	53.5	33.5	Anise oil	[2] T	[2] T
Brown sugar	15.0	15.0			
Corn oil	4.0	4.0	Total	100.0	100.0

[1] The 4.5 percent of minerals was made up 2.5 percent of the Hawk and Oser modification of the Osborne and Mendel mineral mixture (*1*) and 2.0 percent of a mixture of ground limestone 40 parts, steamed bonemeal 40 parts, and salt 20 parts.

[2] T = trace.

As may be noted in table 1, brown sugar was used instead of cane sugar, and corn oil was added instead of lard. This was done in an effort to increase the palatability of the diet. A different mineral mixture also was used. To these diets, a small quantity of anise oil was added. This was done to impart a pleasant and distinct taste, as well as odor, to the diet, which was otherwise deficient in these respects. It was hoped that the addition of this material would aid in securing greater feed consumption. Anise oil can be added readily to any ration, and by using it abrupt changes may be made from one ration to another without very much danger of the lambs refusing the new ration because of the difference in taste or smell.

In order to correct for any possible decrease in the palatability of the mixture that might result from the presence of such a large amount of fine starch, the starch was cooked together with the other constituents of the diet, except the yeast and minerals. This cooked material was thoroughly dried in a drying room and then coarsely ground. Later the minerals and yeast were added to complete the diet.

The diet as fed was a brownish, rather gritty material, somewhat like small, hard seeds. It was sweet to the taste and had a noticeable odor, as well as taste, of the anise oil. From the standpoint of taste, odor, and physical condition, the diet seemed about as palatable as could be devised with the ingredients used. However, no direct test was made to determine whether these modifications actually did improve the palatability of the mixture.

EXPERIMENT 1

For the first experiment a thrifty grade lamb was obtained from the university flock. The lamb was fed a ration of ground corn, linseed meal, and timothy hay for a short time, and then the low-nitrogen diet was gradually substituted for part of the concentrates until the lamb received only the low-nitrogen diet plus the hay. So far as could be determined, the low-nitrogen diet was just as readily consumed as the concentrate mixture. After this substitution, the allowance of the purified diet was increased and the amount of hay was decreased. Within a few days the lamb was receiving only the low-nitrogen diet.

The low-nitrogen diet was eaten readily and remained very palatable for the first 2 days that it was given as the only feed. On the third day, however, the lamb refused some feed and later went badly off feed. The feed consumption was decidedly under the former level, and the lamb became irregular in its eating. The feces were loose and had a bad odor. Without question, the appetite and the digestive processes of the lamb were badly upset after this short period on the low-nitrogen diet.

The question naturally arose as to why the feed, which at first had apparently been eaten with relish, should suddenly become objectionable to the lamb. It was thought that possibly the change to the purified diet had been too abrupt. However, there were no definite indications that such was the case. The most logical explanation appeared to be that the low-nitrogen diet had seriously disturbed the digestive system of the lamb and so had greatly reduced its desire for food.

After a few days, the feeding of the low-nitrogen diet was discontinued and the lamb was again fed the ordinary barn feeds. Although

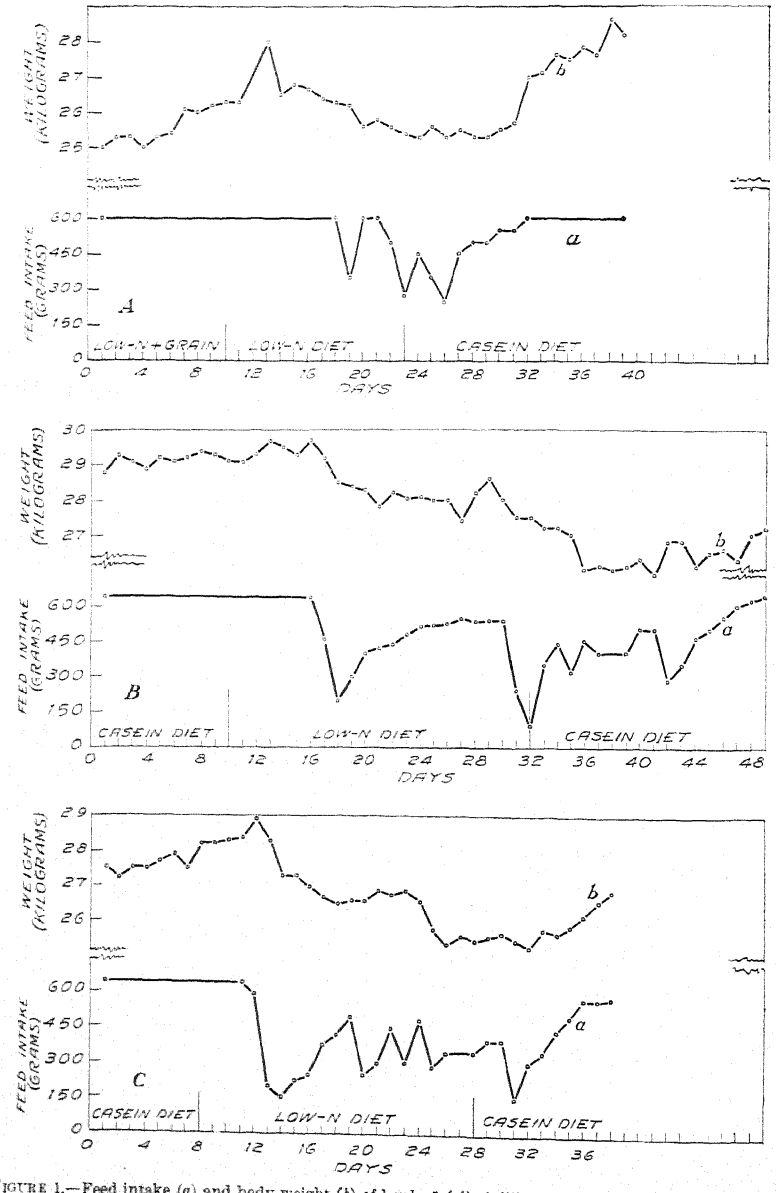


FIGURE 1.—Feed intake (*a*) and body weight (*b*) of lambs 1 (*A*), 2 (*B*), and 3 (*C*) while on the indicated experimental diets.

its improvement was slow in the beginning, by the end of 10 days it was again eating satisfactorily and gaining in weight. Evidently it had completely recovered from the period of feeding on the low-nitrogen diet.

It was then decided to check these results by repeating the procedure with the same lamb. This time, however, instead of decreasing the amount of grain as in the first trial, the amount of hay was decreased as the low-nitrogen diet was added. After a few days, the hay was entirely removed so that the lamb was getting about one-third of the relatively high-protein concentrate mixture (corn and linseed meal) and two-thirds of the low-nitrogen diet. The lamb readily consumed this mixture and made considerable gain in weight. After a period of 10 days, it was fed only the low-nitrogen diet. The effect of this change in diet on the feed consumption and on the daily body weights throughout the experiment are shown in figure 1, *A*.

As figure 1, *A*, *a*, indicates, at the beginning the lamb ate the same amount of the low-nitrogen diet that it had of the diet plus the corn and linseed-meal mixture. Here, again, was evidence that the low-nitrogen diet was at first just as palatable as the natural feeds. At the end of 8 days, however, the lamb refused to clean up its feed. It had the appearance of being in extreme discomfort and was not so active as formerly. The feces lost their typical pellet shape and gave off a strong odor. In practically all respects the lamb had developed the same condition as when previously fed this same diet.

Instead of bringing the lamb back to a normal condition by giving the natural feeds, it was fed a purified diet containing considerable casein. This diet was prepared exactly as the low-nitrogen diet had been except that in this mixture casein was added to the extent of 20 percent of the total diet and the percentage of starch was reduced accordingly. By reason of the cooking and the addition of anise oil, the odor, taste, and physical condition were the same. Even the color was nearly the same, the casein diet being just a trifle darker. No particular difference in the desire for feed was observed when the casein diet was first offered to the lamb, but after a few days, it developed a stronger and more uniform appetite, and at the end of 8 days, it was eating the former amount of feed. The casein diet was fed for 8 additional days, during which time the lamb became normal in all respects and had an excellent appetite for the diet.

The changes in the body weight of the lamb during the experiment are of interest. As shown in figure 1, *A*, *b*, the lamb made unusually large gains during the period in which the concentrate mixture was fed along with the low-nitrogen diet. The extent of these gains was no doubt due in part to the previous emaciated condition of the lamb. The lamb also continued to gain for a few days on the low-nitrogen ration, but after a rather marked increase in weight, it began to lose rapidly. This loss of weight corresponded to the period in which it had diarrhea. Shortly after changing to the casein diet, the lamb improved in body weight, and by the end of the experiment it was making good gains.

EXPERIMENT 2

Experiment 2 was conducted primarily as a check upon the first experiment. While the low-nitrogen diet had proved unsuccessful in two attempts with the same lamb, it was believed desirable to try the

same diet with additional lambs before admitting failure. Also, the behavior of the first lamb on each of the diets had been of especial interest and further observations seemed desirable. All of the rations used in this experiment were the same as those used in experiment 1.

Two grade lambs were secured for this study. They were very similar to the lamb used in the first experiment, but were somewhat heavier in weight. The 20-percent casein diet was gradually substituted for the customary barn ration, and after the lambs had been on the casein diet for a period of time, they were changed to the low-nitrogen diet. The casein diet was fed again at the end of the experiment. The daily feed intake and the daily weights throughout the period are shown in figure 1, *B* and *C*.

The results secured with lambs 2 and 3 were similar to those obtained with lamb 1. The lambs apparently did not notice when the change was made from the casein diet to the low-nitrogen diet. Their feed intake remained the same for a few days and their appetites were good. Then suddenly they began to refuse feed, and for the duration of the period on the low-nitrogen diet, they would not consume the feed at the former level of intake. The condition of their feces was also abnormal.

A change was made back to the casein-containing ration, and, again, insofar as could be determined, the lambs did not observe the change in their feed. After a few days, however, a noticeable improvement was made and both lambs almost completely regained their former level of feed intake and appeared to be in good condition. Considerable increases in body weight were also made.

DISCUSSION

The data presented bring out clearly the fact that there are important factors in rations other than physical appearance, taste, or smell which may determine the amount of a feed or a ration that an animal will consume. Insofar as could be judged by inspection, the casein diet and the low-nitrogen diet were practically identical. The cooking and subsequent grinding of most of the constituents in each diet made them markedly similar in appearance and the taste and odor were almost identical, owing primarily to the addition of the anise oil. The accuracy of these observations is borne out by the fact that in no instance when the diets were changed did the lambs give any indication that they noticed the change. It therefore seems logical to conclude that both diets were palatable and that the difference in feed consumption was due to the presence of the casein or more protein in one of the rations.

The low-nitrogen diet as prepared did contain an appreciable amount of protein furnished by the yeast. While this amount of protein would not have been sufficient to maintain the lambs adequately over a long period, it seems unlikely that the lack of even this essential nutrient would become apparent in so short a time. Probably the poor results obtained on this diet were due to the severe digestive disorders which occurred, rather than to the fact that the lambs were suffering from an actual deficiency of protein.

As indicated by the curves in figure 1, the lambs made irregular increases in body weight at about the time that they began to refuse the low-nitrogen diet. This strongly indicated that normal digestion

followed by the elimination of the undigested material was not taking place. Also, in every instance, soon after the lambs began to refuse feed, they developed diarrhea to some extent.

Daily fecal weights on the dry-matter basis were obtained in the observations with lamb 2. The data during the period on the low-nitrogen diet clearly indicate a decreased excretion of feces during the period prior to going off feed, when the lamb was making a sharp increase in body weight. These conditions were followed by a marked increase in the weight of feces excreted which corresponded to the beginning of the diarrhea experienced by the lamb. All of these results indicate that the digestive system of the animal was badly upset.

While there are no specific data to explain the poor results obtained when the low-nitrogen diet was fed, it is possible that the cellulose in this diet may have been largely responsible for the digestive disorders which occurred. There is a common belief that protein stimulates the development of bacteria in the digestive tract, and if this is true the feeding of the low-nitrogen diet may have resulted in much less bacterial action than normal, causing a less complete digestion of the cellulose. This condition would result in digestive disorders and a decrease in the amount of feed desired by the animals.

SUMMARY

An attempt was made to prepare a low-nitrogen diet for lambs that could be used in connection with some of the studies at this station on the nutritive value of proteins. A mixture which was relatively low in nitrogen and contained only purified ingredients was fed to three lambs. The diet appeared to be very well liked for the first few days of feeding. At the end of that time, however, the lambs ate much less feed and gave definite indications of digestive disorders. Although the feeding of the diet was continued for several days after these conditions developed, the lambs would not consume as much feed as they had readily eaten of a diet which was very similar except that it contained considerable protein. Apparently the digestive disorders brought on by the feeding of the low-protein ration caused the lambs to desire less feed. These results show clearly the effect that a deficiency of protein may have on the digestive system of an animal and on the amount of feed that it will consume.

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CROSS TRANSFER OF MINERAL NUTRIENTS IN THE TOBACCO PLANT [1]

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INTRODUCTION

Uniformity of growth in plants is a matter of considerable theoretical as well as practical interest. In studies concerning the mineral nutrition of tobacco, in regard to distinctive growth effects manifested by this plant (*6*, *7*, *10*, *11*),[2] it was frequently observed under field conditions (*12*) that one-half of a leaf exhibited characteristic deficiency effects when such effects were not so evident on the other half. The observed effects appeared to be due to a failure of cross transfer of nutrients even between halves of an individual leaf. These observations furnished the basis for the investigations reported in this paper. The problem appears to have some bearing on the field placement of fertilizers, particularly separate placements of the different ingredients and deferred side dressings.

The work of Gile and Carrero (*8*, *9*) has definitely shown a reduction in growth and decreased absorption when the roots are divided between different containers so that some portion or all of the root systems are supplied with an incomplete nutrient solution. These authors did not describe in detail the appearance of the plants grown in tests. The studies by Auchter (*2*) with woody plants support the idea that there is little or no cross transfer of nutrients but a free cross transfer of water in woody plants. Auchter found that there was a unilateral distribution, that is, the nitrate applied on one side of the tree produced responses that were evident on the side of the tree to which the application was made. Caldwell (*3*, *4*) concluded that salts and carbohydrates were translocated up or down the plant stem but that there was little cross transfer of materials. Localization of day-length effects reported by Garner and Allard (*5*) further emphasizes the failure of translocation. These workers found striking localization of the principle responsible for the initiation of flowering in plants. These observations are difficult to interpret from the standpoint of plant anatomy if there be any considerable movement through the vascular bundles, since Artschwager (*1*) has reported more or less complete anastomosis of these bundles, which should operate to mix all materials moving through these channels.

[1] Received for publication Jan. 18, 1937; issued October 1937.

[2] Reference is made by number (italic) to Literature Cited, p. 481.

PROCEDURE

In an effort to determine why only one side of a leaf may manifest distinctive symptoms due to a deficiency of any one of the essential elements necessary for growth, the roots of the tobacco plant (*Nicotiana tabacum* L.) were divided into approximately two equal portions, each of which was placed in a 1-liter Pyrex beaker, tall form. The plant was held in position by a varnished wooden board provided with suitable holes and a stake to which the plant was tied. Early attempts to produce the desired result were not successful, apparently because fibrous roots developing near the junction of the beakers tended to mix the two solutions through a wicklike effect. This complication is particularly serious in solution cultures but should not be operative in sand or soil cultures. In an effort to overcome this difficulty in studies conducted during 1930, the stalk was split to about 1 inch above the root crown. Under these conditions it was possible to obtain definite effects, which are described later.

Since the procedure just described is open to the objection that the stele would be divided and cross transfer, therefore, prevented, further studies were undertaken. The fibrous roots were removed to avoid the mixing of solutions that occurred in the early trials. Solutions were prepared like those used in previous studies (*11*), from each of which one of the following elements was withheld in turn: Nitrogen, phosphorus, potassium, calcium, magnesium, sulphur, boron, iron, and manganese. After having been disinfected with silver nitrate according to the procedure previously described (*11*), plants were transferred on April 1, 1936, from soil to duplicate culture solutions from which, in each instance, one of the above elements had been withheld, except that in one group all these elements were included in both containers. Each container was aerated by bubbling with compressed air at approximately 1 liter per hour. On May 17 all solutions were renewed, a complete solution being added to the container for one-half of the roots of each plant of the incomplete groups and to the containers for both halves in the complete group. Additional nutrient was subsequently added at intervals of 2 weeks.

The procedure of growing the plants in incomplete nutrient solutions until characteristic deficiency symptoms developed, as previously described (*11*), was adopted because it appeared to give the most logical approach to the problem. It had been previously determined that the development of symptoms was slower than was the subsequent recovery. Such procedure caused the plant to develop symmetrically in regard to root and top growth, permitting later changes to produce sharper contrasts.

RESULTS AND DISCUSSION

Except in the case of manganese, all cultures were manifesting characteristic deficiency effects at the time provision was made for supplying one-half of the roots with a complete nutrient solution (*11*). Two weeks later it was evident that cross transfer was not rapid enough to give uniform recovery. This condition extended even to a half of the individual leaf, as shown in figure 1, where a large portion of the leaf on one side of the midrib has recovered its normal green color while the remaining half is chlorotic because of a deficient supply of magnesium. This appears to be the first reported instance

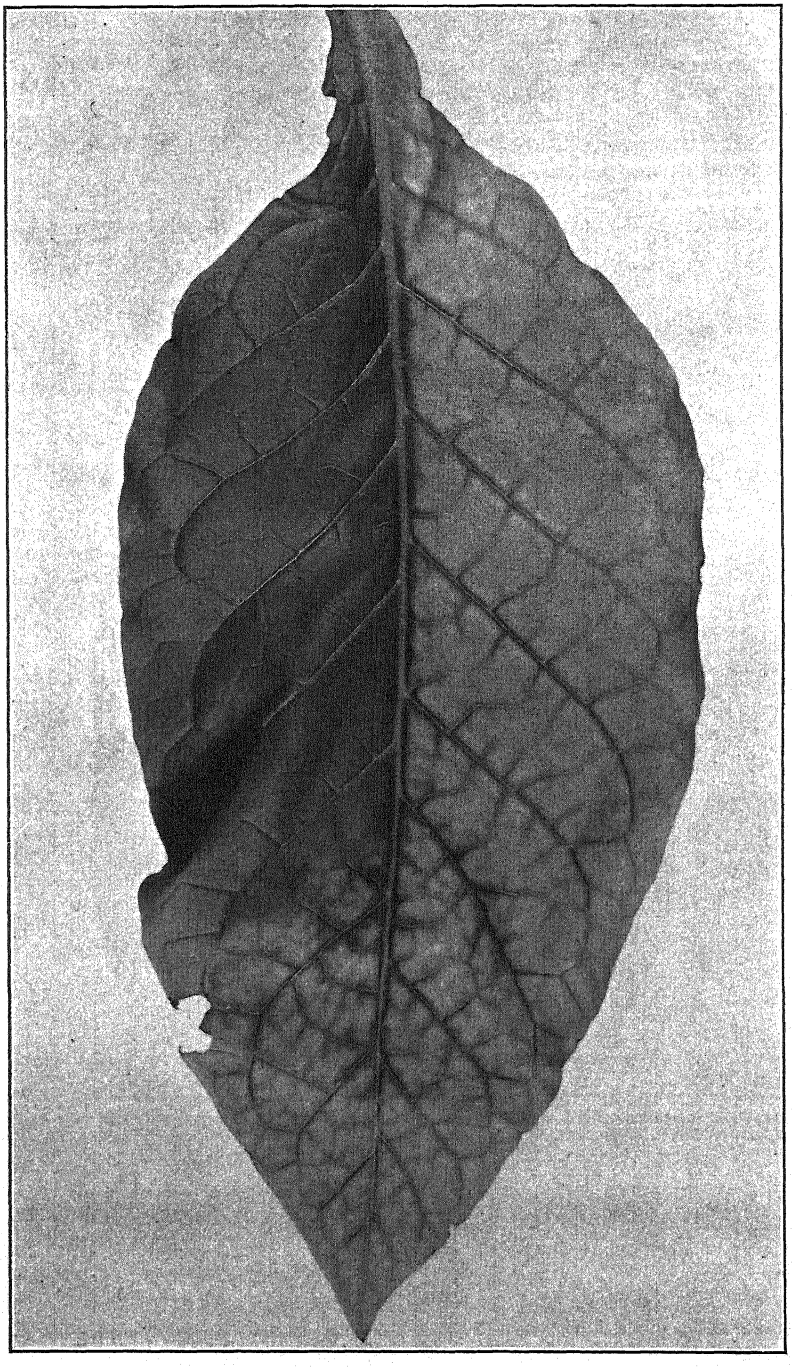


FIGURE 1.—Tobacco leaf manifesting one-sided recovery caused by supplying the element magnesium to one-half of the root system and withholding it from the other half.

where a failure of cross transfer of a mineral nutrient in the individual leaf has been shown to occur. This situation was more or less common to deficiency symptoms manifested for all elements except manganese. Possibly the experiment was not of sufficient duration to induce effects from shortages of this element. The unilateral leaf manifestation was not so commonly observed in calcium and boron deficiency combinations.

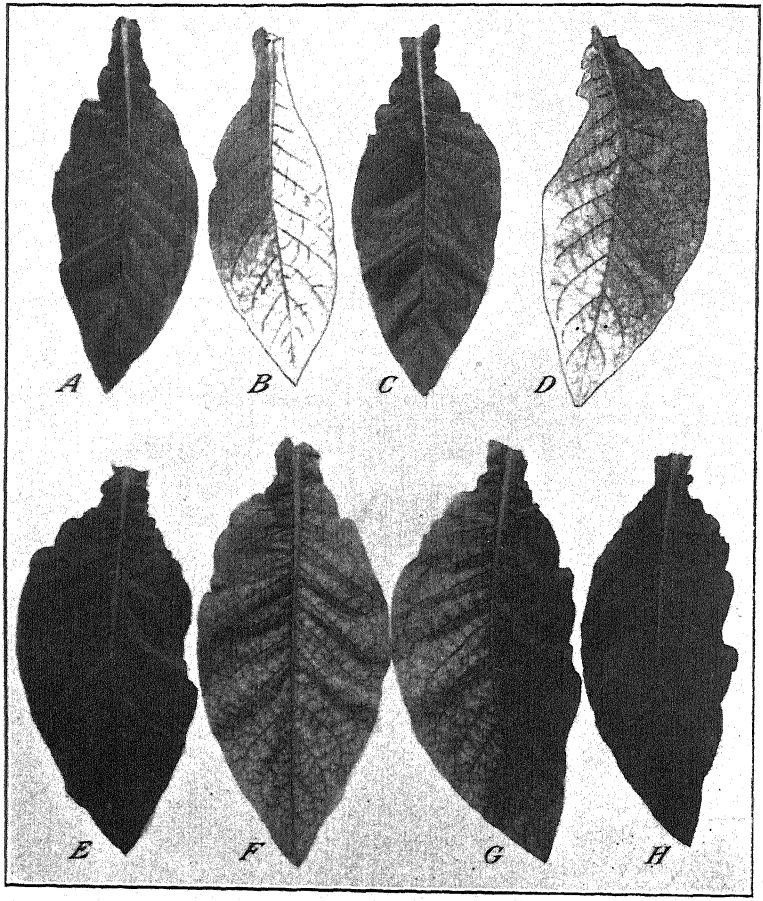


FIGURE 2.—Tobacco leaves showing distribution of recovered and chlorotic leaves (*A–H*) removed from a plant from the base, *A*, upward to *H*, when magnesium was supplied to one-half of the root system and withheld from the other half.

A question of some interest is whether recovery may be unilateral in the individual leaf, but this appears not to be the case. In some instances the entire leaf may show bilateral recovery, or again there may be a leaf that shows no recovery. Nor does there appear to be any definite relationship between the arrangement of affected leaves on the stalk and the 3/8 phyllotaxy of the tobacco plant. These observations are supported by figure 2, in which the leaves *A* to *H* are arranged as they occurred on the stalk, beginning at the base.

In this instance magnesium was supplied to only one-half of the roots. This same observation held true in greater or less degree for all combinations tested. In many instances there does appear to be unilateral or half-leaf manifestation, but the leaf halves concerned do not always occur on the same side. This is shown, for example, in figure 2, for *B* shows recovery on one-half while *D* manifests recovery on the opposite half.

Conceivably it would be possible so to subdivide the roots that there would be a symmetrical effect, but such a result was never

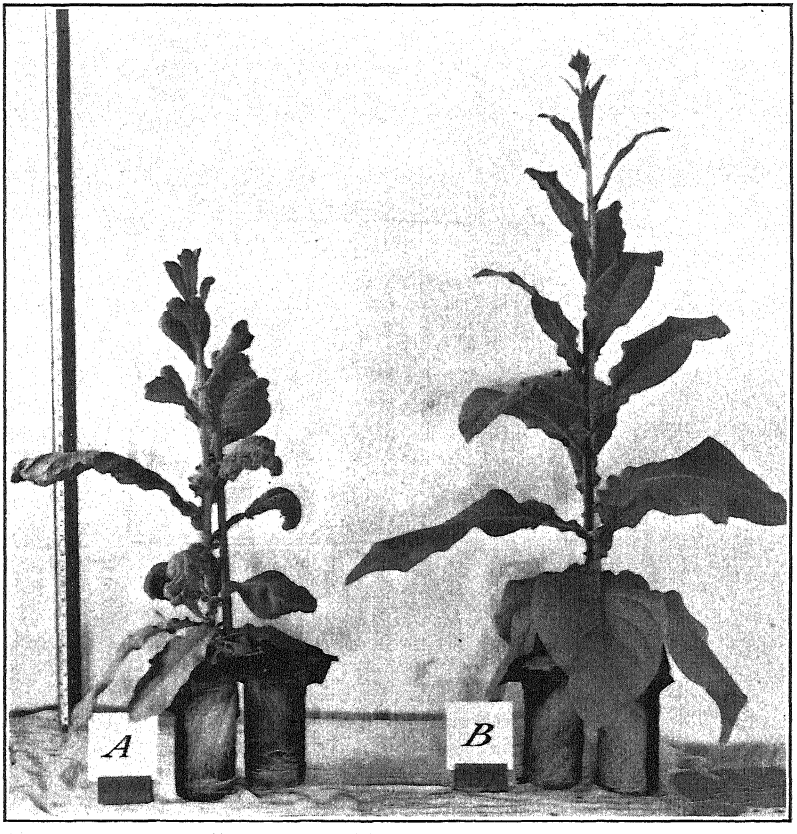


FIGURE 3.—Tobacco plants grown in solution cultures. *A*, Calcium added to container on left after being withheld for a time. This element was not added to container on right for period of observation. *B*, Complete nutrient solution supplied to both containers for the entire period of observation.

observed in the combinations tested. It appears that no systematic arrangement can be expected, since it would be highly improbable that by mere chance the roots could be divided in such a manner as to produce such a result. The plants made additional growth following their transfer to the conditions adapted to study of recovery. This growth also manifested the failure of cross transfer, indicating that there would have been this failure even if the roots had been grown half in complete and half in incomplete solutions for the entire period. The experiment was discontinued June 18, approximately

1 month after the transfer was made, and none of the plants manifested symmetrical growth when any particular element was supplied to only one-half the root system.

Failure of cross transfer of an essential nutrient results in a one-sided or twisted growth, as shown in figure 3, *A*, where calcium was not supplied during the early growth but later was supplied to the half of the root system nearest the letter *A*. It can be observed in this plant that the increase in size of the leaves is not unilateral in its distribution. Complete recovery from symptoms induced by calcium deficiency is not possible, since there is an actual break-down of the meristematic tissues; but it is apparent that certain leaves have increased in size, even though the leaf tissues are missing at the tips and margins. The control plant (*B*), in which calcium has

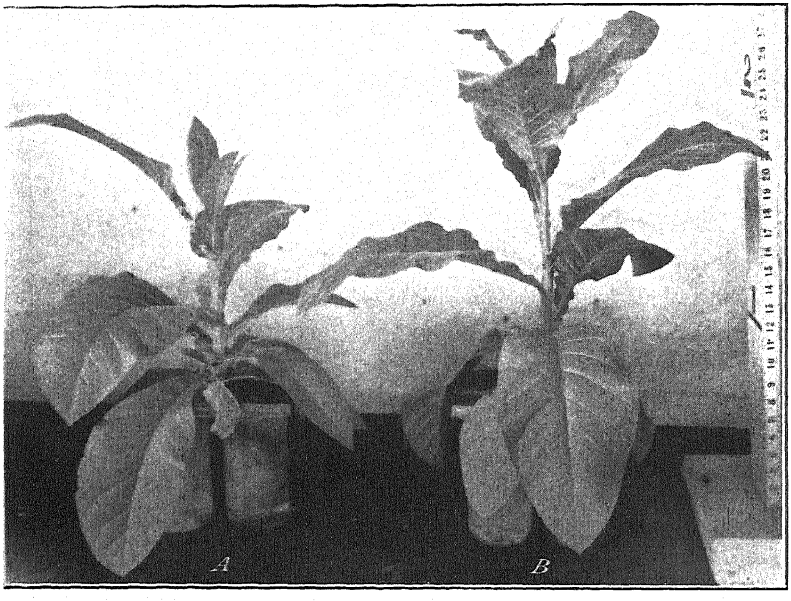


FIGURE 4.—Tobacco plants grown in solution cultures. *A*, Sulphur added to container on left after being withheld for a time. Container on right received no added sulphur. *B*, Complete nutrient solution supplied to both containers.

been supplied to the entire root system during the period of observation, manifests symmetrical development of both root and top growth.

Unilateral distribution of mineral nutrients does appear to occur in some instances, as indicated by symptom manifestations. It was characteristic of those cultures in which sulphur was not supplied for a time and then was added to the container in which one-half of the roots were growing (fig. 4, *A*) that rapid recovery was exhibited on the side of the plant to which the element was supplied. Again, it is evident that the lower leaf on plant *A* manifests a one-sided growth. In figure 4, *A*, the container to the right has received no added sulphur, and it is evident that this half of the plant is stunted. There was also a decided difference in the shade of green, which failed to register in the photograph. The leaves and even the leaf halves that manifested sulphur deficiency were a decidedly lighter

green. The control plant (*B*) is normal in appearance and symmetrical in development.

It has been observed under field conditions (*12*) that leaf halves manifest deficiency symptoms, and it would appear that failure of cross transfer is the explanation. It is conceivable that one portion of the root zone in which the plant is feeding is relatively more deficient in an element than another portion and that, owing to failure of cross transfer in the plant, one-half of the leaf would manifest more pronounced symptoms than the other half. In view of the fact that this situation has been observed to occur under field conditions, studies in fertilizer placement should take into account this phase of the problem. Placement to one side of the row and the application of different ingredients in separate, widely separated bands or positions in relation to the root system of the plant may not be desirable practices. The method of delayed side dressings should include ample provision for adequate distribution in relation to the root zone of the plant in question. The results presented in this paper appear to offer a logical basis for explaining the more efficient action of the fertilizer when placed near the plant in the region of maximum root development.

SUMMARY

It has been possible to reproduce experimentally, on one-half of an individual leaf, symptoms of nitrogen, phosphorus, potassium, magnesium, sulphur, and iron deficiency by withholding the element from a portion of the root system. Failure of cross transfer of the essential nutrients did not always manifest distinctive effects unilaterally on the individual leaf, and the plant in some instances showed an entire leaf normal while an adjacent leaf bilaterally manifested symptoms of deficiency. A twisted or one-sided growth also resulted from deficiency of each of the above-mentioned elements and also of calcium or boron when they were not supplied to the entire root system.

There was not always a unilateral distribution of the symptoms in relation to the root and top growth when the roots were divided so as to supply a given element to one-half of the root system and withhold it from the other half. Depending apparently upon root and top-growth relations, it appears to be merely a matter of chance as to what type of growth manifestation is obtained.

The possible relation of these findings to fertilizer placement under field conditions is pointed out.

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PHYSIOLOGIC RACES OF TILLETIA TRITICI AND T. LEVIS[1]

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INTRODUCTION

Since Faris (*3*)[2] obtained strong indications of physiologic specialization in *Tilletia tritici* (Bjerk.) Wint. and *T. levis* Kühn, pathogenic differences between races of both species have been studied by a number of investigators in the United States and Canada (*1, 2, 5, 6, 7, 8, 9, 10, 12, 13, 14, 15*). In these experiments the methods used have been more or less adapted to local conditions, and this has resulted in a lack of uniformity in the use of differential hosts and in the system of numbering distinct races. Consequently it is to be expected that a number of the physiologic races that have been identified by different investigators will be duplicates. The reports of these experiments do, however, serve as definite evidence that physiologic races of these fungi have become established in the important wheat-growing areas of the United States. The significance of this fact with regard to the problem of bunt control needs no extensive elaboration.

Certain wheat varieties have been distributed in the past that had proved to be resistant to bunt prevalent in the vicinities where the varieties were developed. However, when these varieties were grown commercially they were found to be susceptible. In most instances outbreaks of bunt in such varieties have been definitely associated with the appearance of previously undescribed races of the two species. Obviously, then, if a breeding program for the development of smut-resistant varieties of wheat is to be successful it is essential to know the number, distribution, and prevalence of physiologic races. To obtain this information more rapid progress will be made if a standard system for race identification is developed and generally adopted. The present paper is intended as a first step in this direction.

MATERIALS AND METHODS

Thirty-one collections of *Tilletia tritici* and 34 of *T. levis* have thus far been given race numbers by various investigators in this country. Of this group 24 of the numbered races of the former and 29 of the latter were obtained for testing by the writers. In addition, a number of miscellaneous collections of both species were tested.

Ten varieties each of winter and of spring wheat were used as differential hosts. The winter wheats were Hybrid 128 (C. I.[3] 4512), Ridit (C. I. 6703), Oro (C. I. 8220), Albit (C. I. 8275), Hohenheimer (C. I. 11458), Hussar (C. I. 4843), Minhardi (C. I. 5149), White Odessa (C. I. 4655), Martin (C. I. 4463), and Turkey (C. I. 6175). In the final analysis of data only the first six of these were retained as differential hosts. The other four were not considered for the following reasons: Minhardi was found to be similar to Hybrid 128 in its reaction to all races and collections tested; White Odessa was similar to

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[2] Reference is made by number (italic) to Literature Cited, p. 495.

[3] C. I. denotes accession number of the Division of Cereal Crops and Diseases (formerly Office of Cereal Investigations).

Albit in this respect; no pathogenically distinct races could be differentiated on Martin that were not equally well differentiated on either Albit or Hussar; Turkey (C. I. 6175) was not consistent in its reaction to certain races when grown under a fairly wide range of environmental conditions. The spring wheats were Ulka (C. I. 11478), Marquis (C. I. 3641), Canus (C. I. 11637), Mindum (C. I. 5296), Hope × Ceres (C. I. 11432), Ruby (C. I. 6047), Garnet (C. I. 8188), Thatcher (C. I. 10003), Golden Ball (C. I. 11477), and Vernal emmer (C. I. 3686). In the analysis of data only the first four of these were retained as differential hosts. Certain races could be identified by their reaction on the latter six varieties, but none that were not equally well differentiated on the Ulka, Marquis, Canus, and Mindum combination.

Seed of the differential hosts was treated with formaldehyde according to the standard dip method, thoroughly washed with water to remove all traces of formaldehyde, and allowed to dry. Powdered inoculum from each collection was then applied to each lot of seed at the rate of approximately 0.5 g to 100 g of seed. The inoculated seed was planted at the rate of 5 g to the row in duplicate systematically distributed 6-foot rows. The inoculum of each race to be used in the succeeding year's test was collected on the same date and subsequently stored at room temperature in the laboratory. In preparing the inoculum and in inoculating and planting the seed, adequate precautions were observed to prevent mixing of the spores of different species and races. The smut percentages obtained were based on counts of 300 heads per row at Bozeman, Mont., and counts on the total number of heads per row at Kearneysville, W. Va., and Pullman, Wash. There were approximately 200 heads per row at Kearneysville and 400 per row at Pullman.

In the analysis of these data only those collections of smut possessing fairly wide differences in pathogenicity were considered as distinct races. With the present technique for separating races of *Tilletia*, environmental conditions are not accurately controlled nor are they duplicated at different stations and in different years. It is therefore impossible to make such fine distinctions as have been made in the rust fungi, in the study of which the environmental conditions affecting both the host plant and the fungus may be easily controlled. When a sufficiently large number of tests have been made under controlled conditions or under different environmental conditions in sufficient number to permit a statistical analysis of the results, smaller differences in pathogenicity may possibly be found to be significant. Until then, classifications of races of *Tilletia* are necessarily more or less arbitrary, and it would seem logical that separations should be made only where the differences in pathogenicity are fairly wide and reasonably consistent. In previous work relatively narrow limits in infection range classes have been considered significant for the purpose of separating physiologic races. As a result, four to six classes have been used by various investigators. However, in the present tests variations between results with the same smut collections at different stations in 1 year and in the results from replications at the same station were frequently great enough to interfere with the use of a classification with more than three infection classes as follows:

0–10 percent infection = resistant class (R).
11–40 percent infection = intermediate class (I).
41–100 percent infection = susceptible class (S).

In establishing these classes consideration has been given to the tendency toward variation in smut percentages produced by the same races on the same varieties, in different replications, in different years, and in different localities. It is clear from the data in table 1 that within the resistant class the variability in percentages of infection is small and that the upper limit for the class should be approximately 10 percent. These data also indicate that as the degree of resistance decreases the variability in the percentages of infection obtained with any one collection of smut increases. For example, as recorded in table 1, the percentage of smut on Albit inoculated with T–4 varied from 10 to 44 percent depending on the locality or the year the test was made. It is to allow for such variations in percentages as these that the limits for the intermediate and susceptible classes have been established.

Tests for the differentiation of physiologic races are considered satisfactory in these experiments only when 60 percent or more smut developed in the generally susceptible varieties, Hybrid 128 and Ulka. Accordingly no data are presented from tests in which infection in these varieties failed to reach this figure. The classification as recorded is based on results obtained in tests with winter wheat in 1 of the 4 years the experiments were made at Kearneysville, W. Va.; in 1 of the 3 years at Bozeman, Mont.; and both of the 2 years at Pullman, Wash. In the tests with spring wheat, satisfactory data for race differentiation were obtained in 1 of the 3 years tests were made at Bozeman, and 1 of the 2 years at Pullman. No significant data were obtained in two seasons the tests were made with spring wheat at St. Paul, Minn., because of drought conditions, and with winter wheat at Corvallis, Oreg., because of poor stands.

Reactions that differentiate physiologic races are indicated in table 3 by the bold-faced type, and the letter T or L is prefixed to the race numbers to denote the smut species *Tilletia tritici* and *T. levis*, respectively.

RESULTS

IDENTIFICATION OF PHYSIOLOGIC RACES

At the present time tests adequate to warrant classification have been made with 24 collections of *Tilletia tritici* and 28 of *T. levis*. From these collections 11 pathogenically distinct races of *T. tritici* and 8 of *T. levis* have been identified and assigned letters and numbers T–1 to T–11 and L–1 to L–8, respectively. The average percentages of smut produced by these races at different stations and in different years are recorded in table 1. The sources of inoculum for these tests, together with the assigned race numbers, are recorded in table 2. The reactions that serve to differentiate the races of *T. tritici* and *T. levis* are presented in table 3.

In previous investigations the identification of physiologic races of *Tilletia tritici* and *T. levis* has been based upon their reaction on either winter or spring wheats alone. The results of the present studies emphasize the value of using both winter and spring varieties as differential hosts. For example, races L–1, L–2, and L–3 (table 3) are differentiated on spring wheats alone, races L–6 and L–8 on winter wheats alone, and races L–4 and L–5 on a combination of both winter and spring wheat varieties. Race L–7 is clearly differentiated by its reaction on either the spring or winter wheat varieties. Similar results were obtained with races of *T. tritici* (table 3).

TABLE 1.—*Percentages of bunt obtained in 6 varieties of winter wheat and 4 varieties of spring wheat inoculated with 11 physiologic races of Tilletia tritici and 8 physiologic races of T. levis at Bozeman, Mont., Pullman, Wash., and Kearneysville, W. Va., 1934–36*

TILLETIA TRITICI

Physiologic race no.	Year tested	Percentage of bunt in varieties at localities indicated																			
		Hybrid 128 (C. I. 4512)				Ridit (C. I. 6703)				Oro (C. I. 8220)				Hohenheimer (C. I. 11458)				Hussar (C. I. 4843)			
		Bozeman	Pullman	Kearneysville	Average	Bozeman	Pullman	Kearneysville	Average	Bozeman	Pullman	Kearneysville	Average	Bozeman	Pullman	Kearneysville	Average	Bozeman	Pullman	Kearneysville	Average
T–1	1934	69				2				4				1				5			
	1935		82	84	80		2.0	5	3		2.0	1	2		1.0	0	1.0		0.4	1.0	2.0
	1936		85				3.0				1.0				.0				1.0		
T–2	1934	64				0				0				0				2			
	1935		81	95	84		.0	5	2		.2	1	1		.3	0	.1		.3	.3	1.0
	1936		93				1.0				2.0				.0				.0		
T–3	1934	75				2				4				1				3			
	1935		92	86	86		8.0	4	7		9.0	2	4		.0	0	.3		1.0	.0	1.0
	1936		92				12.0				1.0				.0				.2		
T–4	1934	90				1				4				1				3			
	1935		93	90	91		3.0	5	3		5.0	2	3		.0	0	.3		1.0	.4	1.0
	1936		92				2.0				1.0				.0				.0		
T–5	1934	87				9				8				0				9			
	1935		91	93	91		10.0	6	8		7.0	3	5		.0	2	1.0		2.0	3.0	4.0
	1936		93				5.0				3.0				.0				1.0		
T–6	1934	71				2				3				1				5			
	1935		91	94	82		1.0	3	2		3.0	1	2		.0	0	.3		5.0	.4	3.0
	1936		71				2.0				1.0				.0				.4		
T–7	1934	79				3				2				2				26			
	1935		90	81	83		5.0	1	3		3.0	2	2		.0	0	1.0		30.0	42.0	33.0
	1936																				
T–8	1934	78				8				2				3				34			
	1935		95	86	89		5.0	6	6		5.0	3	4		.0	1	1.0		57.0	78.0	53.0
	1936		95				5.0				5.0				.0				42.0		
T–9	1934		90				2.0				3.0				34.0				.0		
	1935				87				1				2				32.0				1.0
	1936		84				.1				1.0				29.0				1.0		
T–10	1934		91				1.0				5.0				69.0				C		
	1935		86		90		1.0		1		2.0		3		60.0		67.0		.0		.0
	1936		93				2.0				2.0				73.0				.0		
T–11	1934																				
	1935		90		84		55.0		56		1.0		2		.0		.0		1.0		3.0
	1936		78				56.0				3.0				.0				4.0		

Physiologic race no.	Year tested	Percentage of bunt in varieties at localities indicated															
		Albit (C. I. 8275)				Ulka (C. I. 11478)			Marquis (C. I. 3641)			Canus (C. I. 11637)			Mindum (C. I. 5296)		
		Bozeman	Pullman	Kearneysville	Average	Bozeman	Pullman	Average	Bozeman	Pullman	Average	Bozeman	Pullman	Average	Bozeman	Pullman	Average
T-1	1934	0															
	1935		0.4	0	0.1	73	95	84	33	38	36	0.4	1.0	1.0	18	1	10
	1936		.0														
T-2	1934	1															
	1935		.0	2	1.0	83	94	89	1	2	2	.2	1.0	1.0	74	66	70
	1936		.1														
T-3	1934	0															
	1935		1.0	1	1.0	72	98	85	58	46	52	50.0	46.0	48.0	18	14	16
	1936		.0														
T-4	1934	13															
	1935		18.0	44	21.0	92	91	92	57	39	48	6.0	.0	3.0	22	3	13
	1936		10.0														
T-5	1934	16															
	1935		22.0	13	14.0	86	96	91	71	48	60	51.0	54.0	53.0	22	12	17
	1936		3.0														
T-6	1934	70															
	1935		90.0	92	78.0	92	85	89	41	42	42	3.0	.0	2.0	25	3	14
	1936		58.0														
T-7	1934	69															
	1935		89.0	87	82.0	92	97	95	52	56	54	21.0	15.0	18.0	26	9	18
	1936																
T-8	1934	79															
	1935		88.0	92	88.0	70	91	81	46	46	46	52.0	43.0	48.0	25	7	16
	1936		91.0														
T-9	1934		.0														
	1935				4.0	93	88	91	28	29	29	1.0	.2	1.0	27	23	25
	1936		7.0														
T-10 [1]	1934		4.0														
	1935		.3		2.0			10			33			.1			8
	1936		.1			11	8		44	21		.0	.1		11	5	
T-11	1934																
	1935		2.0		7.0	19	32	26	46	36	41	57.0	46.0	52.0	39	10	25
	1936		11.0														

[1] The reaction of this race on the spring wheat host testers was obtained in 1936 only.

TABLE 1.—*Percentages of bunt obtained in 6 varieties of winter wheat and 4 varieties of spring wheat inoculated with 11 physiologic races of Tilletia tritici and 8 physiologic races of T. levis at Bozeman, Mont., Pullman, Wash., and Kearneysville, W. Va., 1934–36*—Contd.

TILLETIA LEVIS

Physiologic race no.	Year tested	Percentage of bunt in varieties at localities indicated																			
		Hybrid 128 (C. I. 4512)				Ridit (C. I. 6703)				Oro (C. I. 8220)				Hohenheimer (C. I. 11458)				Hussar (C. I. 4843)			
		Bozeman	Pullman	Kearneysville	Average	Bozeman	Pullman	Kearneysville	Average	Bozeman	Pullman	Kearneysville	Average	Bozeman	Pullman	Kearneysville	Average	Bozeman	Pullman	Kearneysville	Average
L-1	1934	82				2				2				3				4			
	1935		92	89	87		3.0	1	2		3.0	1	2		0.0	0	1.0		1.0	0.0	1.0
	1936		83				1.0				.3				.0				0		
L-2	1934	85				2				1				1				1			
	1935		92	89	88		4.0	3	3		2.0	1	2		.0	2	1.0		.0	.0	.3
	1936		86				2.0				2.0				.4				1		
L-3	1934	75				4				2				0				3			
	1935		89	92	87		4.0	3	4		7.0	2	5		.0	0	.0		.0	.3	1.0
	1936		92				5.0				9.0				.0				1.0		
L-4	1934	87				1				5				0				3			
	1935		96	83	87		5.0	3	3		5.0	7	5		.0	0	.0		13.0	2.0	5.0
	1936		81				3.0				1.0				.0				1.0		
L-5	1934																				
	1935		88	83	86		5.0	4	5		7.0	5	5		2.0	0	1.0		4.0	2.0	2.0
	1936		87				6.0				3.0				0				1.0		
L-6	1934	78				5				5				1				11			
	1935		93	89	87		5.0	4	4		7.0	3	6		.0	0	.3		19.0	22.0	15.0
	1936		88				2.0				7.0				.0				6.0		
L-7	1934	63				3				6				0				39			
	1935		85	95	84		4.0	3	5		7.0	9	8		.0	0	.0		26.0	81.0	50.0
	1936		91				8.0				10.0				.0				54.0		
L-8	1934	74				3				56				0				3			
	1935		95	92	89		7.0	10	6		78.0	67	73		.0	0	.0		1.0	2.0	2.0
	1936		95				4.0				90.0				.0				1.0		

Physiologic race no.	Year tested	Percentage of bunt in varieties at localities indicated															
		Albit (C. I. 8275)				Ulka (C. I. 11478)			Marquis (C. I. 3641)			Canus (C. I. 11637)			Mindum (C. I. 5296)		
		Bozeman	Pullman	Kearneysville	Average	Bozeman	Pullman	Average	Bozeman	Pullman	Average	Bozeman	Pullman	Average	Bozeman	Pullman	Average
L–1	1934	0															
	1935		0.3	0.4	0.2	73	96	85	34	39	37	1	0.0	1.0	23	20	22
	1936		.0														
L–2	1934	10															
	1935		10.0	4.0	7.0	89	95	92	60	47	54	4	.1	2.0	27	13	20
	1936		5.0														
L–3	1934	0															
	1935		1.0	.0	.3	90	96	93	42	39	41	62	61.0	62.0	26	6	16
	1936		.0														
L–4	1934	83															
	1935		90.0	86.0	87.0	81	96	89	42	36	39	3	.3	2.0	23	6	15
	1936		88.0														
L–5	1934																
	1935		68.0	73.0	72.0	89	97	93	44	47	46	62	52.0	57.0	22	30	26
	1936		76.0														
L–6	1934	28															
	1935		73.0	79.0	51.0	82	96	89	54	33	44	60	56.0	58.0	21	8	15
	1936		24.0														
L–7	1934	65															
	1935		72.0	87.0	79.0	82	89	86	40	26	33	53	48.0	51.0	40	10	25
	1936		91.0														
L–8	1934	1															
	1935		1.0	.0	1.0	72	95	84	36	43	40	60	69.0	65.0	16	12	14
	1936		.1														

TABLE 2.—*Previously numbered physiologic races and miscellaneous collections of Tilletia tritici and T. levis grouped to correspond with new race numbers*

Smut species	Physiologic race no.[1]	Source of inoculum
Tilletia tritici	T-1	Gaines' 1; Flor's 6; Reed's 3; collection from Davis, Calif.
	T-2	Holton's Vernal emmer race; Holton's Mindum race; collection from Langdon, N. Dak.
	T-3	Reed's 5; Young's Montana collection 38.
	T-4	Reed's 2.
	T-5	Reed's 4.
	T-6	Gaines' 3; Bressman's 7 and 10; Young's Montana collection 20; Rodenhiser and Stakman's Manitoba collection.
	T-7	Collection from Pullman, Wash.
	T-8	Gaines' 2; Bressman's 9; Young's Montana collection 100.
	T-9	Bressman's 8; Flor's 7.
	T-10	Collection from Lind, Wash.
	T-11	Collection from Pullman, Wash.
Tilletia levis	L-1	Reed's 4; Flor's 1, 2, and 6.
	L-2	Rodenhiser and Stakman's 2; Holton's Marquis race; Aamodt's 2; collections from Brookings and Redfield, S. Dak.; Fargo and Dickinson, N. Dak.
	L-3	Gaines' 5; Bressman's 3; Flor's 3 and 5; Melchers' 1 and 6; Young's Montana collection 8; Tingey's Utah collection 2; collections from Bozeman and Moccasin, Mont.
	L-4	Bressman's 4; Tingey's Utah collection 6.
	L-5	Melchers' 2.
	L-6	Collection from Moore, Mont.
	L-7	Flor's 4; Tingey's Utah collection 4.
	L-8	Bressman's 6.

[1] Additional races have been tested that were identified by earlier investigators on different hosts not available for these tests. It may be of interest to record their reaction on the present host testers. Aamodt's races 3 and 4 of *Tilletia levis* had the same reaction as his race 2, reclassified here as L-2. Likewise, Melchers' race 7 was the same as his race 2, reclassified here as L-5.

TABLE 3.—*Relative susceptibility*[1] *of 10 differential hosts to 11 physiologic races of Tilletia tritici and 8 physiologic races of T. levis*

[R=0–10 percent; I=11–40 percent; S=41–100 percent]

TILLETIA TRITICI

Physiologic race no.	Hybrid 128 (C. I. 4512)	Ridit (C. I. 6703)	Oro (C. I. 8220)	Hohenheimer (C. I. 11458)	Hussar (C. I. 4843)	Albit (C. I. 8275)	Ulka (C. I. 11478)	Marquis (C. I. 3641)	Canus (C. I. 11637)	Mindum (C. I. 5296)
T-1	S	R	R	R	R	R	S	[2]**I**	R	**R**
T-2	S	R	R	R	R	R	S	**R**	R	**S**
T-3	S	**R**	R	R	**R**	R	S	S	**S**	I
T-4	S	R	R	R	R	**I**	S	S	**R**	I
T-5	S	R	R	R	R	**I**	S	S	**S**	I
T-6	S	R	R	R	**R**	**S**	S	S	R	I
T-7	S	R	R	R	**I**	**S**	S	S	I	I
T-8	S	R	R	R	**S**	**S**	S	S	S	I
T-9	S	R	R	**I**	R	**R**	S	I	R	I
T-10[1]	S	R	R	**S**	R	**R**	R	I	R	R
T-11	S	**S**	R	R	R	R	I	S	**S**	I

TILLETIA LEVIS

L-1	S	R	R	R	R	R	S	**I**	**R**	I
L-2	S	R	R	R	R	R	S	**S**	**R**	I
L-3	S	R	R	R	R	R	S	**S**	**S**	I
L-4	S	R	R	R	R	**S**	S	I	**R**	I
L-5	S	R	R	R	**R**	**S**	S	S	**S**	I
L-6	S	R	R	R	**I**	**S**	S	S	S	I
L-7	S	R	R	R	**S**	**S**	S	I	S	I
L-8	S	R	**S**	R	R	**R**	S	S	S	I

[1] The reaction of spring wheat differential hosts to this race was obtained in 1936 only, and the results are therefore not strictly comparable with those recorded for the other races. The results are included, however, to indicate particularly the resistance of Ulka to race T-10.

[2] Reactions that differentiate physiologic races are indicated by bold-face type.

The results indicate further that certain varieties, completely susceptible to a large number of races, may carry factors for resistance to others. The spring wheat variety Ulka was originally selected as the susceptible check on which to base the relative susceptibility of the differential hosts. It was found in later tests that Ulka, although completely susceptible to most races, carries factors for resistance to T–10 and T–11. It is possible, then, to strain out certain races from mechanical mixtures even on what has been generally considered a susceptible variety. The races T–10 and T–11 should be noted also with regard to their reaction on the varieties Hohenheimer and Ridit. Although lacking in factors for pathogenicity on Ulka, T–10 is particularly virulent on Hohenheimer, which is highly resistant to all other races except T–9. Hohenheimer is intermediate in its reaction to T–9. Likewise T–11, although relatively low in virulence on Ulka, is extremely pathogenic on Ridit, which is resistant to all other known races.

As indicated in table 2, a number of races which had previously been identified were found to be duplicates. Furthermore, a few smut collections that had been reported as pathogenically distinct did not appear to be so in these tests. Two explanations may be offered for these latter results. Either there were mixtures of races of the same species in the original collections that were later separated by "varietal screening" (*2*, *4*, *17*) or the chlamydospores were of hybrid origin. Usually in making collections 50 or more bunted heads are collected in each field, and within these collections from single fields mixtures of *Tilletia tritici* and *T. levis* are frequently found. This is particularly true of collections made in the western half of the hard red spring wheat area and in the intermountain area of the Pacific Northwest. Pathogenically distinct races also appear to be equally well distributed in these areas, and it would be surprising if some of the original collections did not contain race mixtures. Since the time the inoculum used in the present tests was obtained from the original investigators there has been opportunity for varietal screening of races, for each year inoculum for subsequent tests has been collected from a single differential host. Assuming there were race mixtures in the original collection this would tend to purify the inoculum, and consequently results comparable with those reported originally would not be obtained. Hybridization might also account for the inconsistencies referred to. Relatively little is known regarding the genetics of the bunt fungi; but Flor (*4*) has shown that interspecific and intraspecific hybridization may take place in *Tilletia*, so there is a possibility that the chlamydospores of the original collection were of hybrid origin. Segregates carrying different factors for pathogenicity may have been separated in the process of varietal screening, which would likewise account for the differences in the reactions obtained with the original collection and those in the present tests.

One of the principal objects in standardizing the race numbers and in identifying new races has been to utilize them in the breeding program for the development of smut-resistant varieties of wheat. It is probable that with the present technique for differentiating these races of *Tilletia* minor factors governing pathogenicity are being overlooked.

When new differential hosts are obtained and when tests are made under controlled environmental conditions not only during the time of infection but also during subsequent plant growth, more of these "border-line" races may be differentiated. However, from the practical point of view, in the development of smut-resistant varieties from the present stock of parental wheats it would seem unnecessary to have to take into account races having these minor differences. For example, from the data recorded in table 1, Oro is shown to be resistant to all races except L–8 while Ridit is resistant to all except T–11. It would be expected, then, that certain progeny from Oro × Ridit would carry factors for resistance to all races. Similarly, as indicated in the data in table 1, resistant progeny should be obtained in crosses of Oro or Ridit with Hussar and Hohenheimer.

Although in this paper emphasis has been placed on the separation of races on the basis of pathogenicity, in studying the inoculum of different collections and the effect on the host plant it was obvious that there were other criteria by which a number of races could be identified. They include differences in the size, shape, and hardness of the smut balls, in the degree of reticulation of the chlamydospore walls, in the color of the chlamydospore mass, in the degree to which certain varieties of wheat are stunted, in the partial smutting of certain varieties, in the degree to which the various races cause dropping of the awns of the variety Ulka and, lastly, in the degree to which they cause laxness in the spikes of infested plants, particularly when grown under greenhouse conditions. Certain of these differences were noticed not only between the races found to be pathogenically different but also between collections which, with the present technique, were found to be pathogenically the same.

REACTION OF VARIETIES UNDER DIFFERENT ENVIRONMENTAL CONDITIONS

Soil-temperature and moisture conditions during the time infection may take place (*11*) and post-infection temperatures (*16*) have been found to be some of the factors determining the amount of smut that may develop in a susceptible variety. Furthermore, Aamodt (*1*) reported that when subjected to different temperatures some physiologic races appeared to respond differently in infection capability. Although the plantings in the present experiments were made at the different stations when soil-temperature and moisture conditions were believed to be optimum for infection, there obviously would be other environmental conditions that would not be constant, such as soil type, fertility, and weather conditions after emergence of the seedlings. The question arises, then, as to the constancy of the reaction of the differential hosts to the physiologic races at different stations and in different years. The average percentages of smut produced by the 11 races of *Tilletia tritici* and the 8 races of *T. levis* at different stations and in different years are recorded in table 1. These data indicate a fairly close correlation in the reaction of each race on all of the winter wheat differential hosts at Pullman, Bozeman, and Kearneysville and in the reaction of three of the four spring wheats at Bozeman and Pullman. There are some exceptions. In 1934, Albit, inoculated with T–4, developed only 13 percent of smut at Bozeman; in 1935, 18 percent at Pullman; and in this same year 44 percent at Kearneysville. Likewise, there was a wide difference in the susceptibility of Hussar to race L–7 at different stations. In 1935 this variety inocu-

lated with L–7 developed 26 percent of smut at Pullman and 81 percent at Kearneysville. It is possible that some varieties may differ in their susceptibility to certain races of *T. tritici* and *T. levis* when grown under different environmental conditions. There is evidence that this may be the explanation for the above-mentioned variations. As indicated in table 1, Mindum was found to be generally more susceptible to certain races at Bozeman than at Pullman. In the same tests, however, the pathogenicity of these races did not vary appreciably on other differential hosts. This would indicate that the variation in susceptibility of Albit, Hussar, and Mindum mentioned above is due to the effect of environment on the host rather than on the fungus. Furthermore, the environmental effect appears to be expressed only in the reaction of certain varieties to certain physiologic races. Additional evidence of this kind is presented in table 4. Turkey (C. I. 6175) was found to be decidedly less susceptible to races T–1, T–4, and T–6 and to L–1, L–2, and L–4 at Kearneysville than at Pullman. The reaction with the other races on this variety were, however, clearly more consistent.

TABLE 4.—*Reaction of Turkey (C. I. 6175) and Hybrid 128 (C. I. 4512) to each of 8 physiologic races of Tilletia tritici and T. levis at Kearneysville, W. Va. and Pullman, Wash., in 1935*

Physiologic race of *T. tritici*	Percentage of smut at localities indicated on—				Physiologic race of *T. levis*	Percentage of smut at localities indicated on—			
	Turkey		Hybrid 128			Turkey		Hybrid 128	
	Kearneysville	Pullman	Kearneysville	Pullman		Kearneysville	Pullman	Kearneysville	Pullman
T–1	7	20	84	82	L–1	5	48	89	92
T–2	25	25	95	81	L–2	16	77	89	92
T–3	84	93	86	92	L–3	72	84	92	89
T–4	13	44	90	93	L–4	10	48	83	96
T–5	87	90	93	91	L–5	71	80	83	88
T–6	5	30	94	91	L–6	74	89	89	93
T–7	72	49	81	90	L–7	91	82	95	85
T–8	87	85	86	95	L–8	85	89	92	95

Obviously, this evidence that certain varieties may differ in response to races of *Tilletia* under different environmental conditions is of significance in connection with the problems discussed in this paper. In order to establish the principle of physiologic specialization within the species or to obtain information with regard to the number of pathogenically distinct races that may be present in a somewhat localized wheat-growing area it would seem justifiable to make tests at a single station. However, to more accurately standardize a general system of classification of races and to determine their significance in a general wheat-improvement program, the virulence of races must be determined in comparative tests made in more than one geographical area.

DISCUSSION AND CONCLUSIONS

Eleven physiologic races of *Tilletia tritici* and eight of *T. levis* have been separated on the basis of differences in their pathogenicity on six varieties of winter wheat and four varieties of spring wheat. Although sufficient data are not yet available to permit a detailed

discussion of the distribution of these races, there is evidence that several of the same races commonly found in the Pacific Northwest are also present in the hard red spring and winter wheat areas. With the interchange of seed and the opportunity for wind dissemination of inoculum it will not be surprising if all the identified races eventually become distributed in all of the wheat-growing areas of the United States. Thus, it follows that the development of a smut-resistant variety of wheat is not a localized problem that may be solved by the development of a variety of wheat resistant to smut collected in the immediate vicinity of the station where the breeding work is done. In order to thoroughly determine the bunt resistance of varieties, the facts emphasize the need of extensive tests conducted in widely separated areas with all of the known physiologic races of the bunt fungi. Obviously, the adoption of a standard system of determining the number and distribution of physiologic races will aid in the progress of this program.

The number of pathogenically distinct races that may be identified from a large number of smut collections depends to a considerable extent on the varieties used as differential hosts and on the limits of the infection classes, which up to the present time have been more or less arbitrarily selected. The races to which numbers have been assigned very probably do not represent the total among the several hundred collections of both species of *Tilletia* that were originally made. In fact, in preliminary tests with recently made smut collections there is evidence of additional races some of which have fairly wide differences in pathogenicity. Furthermore, data were obtained on the pathogenicity of a number of collections not recorded here because of their so-called border-line differences in pathogenicity. However, for the time being, in the program for the development of smut-resistant varieties of wheat, we are particularly interested in those races that have rather wide differences in pathogenicity.

From the results of these experiments it seems that it should be possible to breed varieties of wheat possessing desirable agronomic characteristics and a high degree of resistance to the known races of *Tilletia*. Oro and Ridit are both agronomically desirable wheats and are in commercial production at the present time. Oro is resistant to all of these races except L–8 and Ridit to all except T–11. It would be reasonable to expect certain progeny from Oro × Ridit to carry factors for resistance to all of the known races. Likewise, Hohenheimer and Hussar should be of value as parent stocks in the development of smut-resistant varieties. Hohenheimer, though not a commercially desirable wheat, is susceptible to only one race and intermediate in its reaction to another. Hussar, which is at present being used as a parent for both winter and spring wheat crosses, is susceptible to two races and intermediate in its reaction to two. It seems possible that segregates will be obtained from crosses with some of these varieties in which desirable agronomic characteristics are combined with smut resistance to the known races of *Tilletia*.

SUMMARY

Twenty-four collections of *Tilletia tritici* and 29 of *T. levis*, each of which had been identified as a distinct physiologic race by previous investigators, together with a number of miscellaneous collections of both species, have been tested under comparable conditions at several experiment stations. Under the conditions of these experiments a number of the collections appeared to be duplicates. Eleven physiologic races of *T. tritici* and eight of *T. levis* were pathogenically distinct and have been assigned letters and race numbers T–1 to T–11 and L–1 to L–8, respectively.

The value of using both winter and spring wheat varieties as differential hosts is emphasized. With the varieties used in these experiments certain races may be differentiated on winter wheats alone, some by their reaction on spring wheats, and certain others by their reaction on the combination of both winter and spring varieties.

The spring wheat variety Ulka, although completely susceptible to most races, carries factors for resistance to at least two races. It is possible, then, to strain out certain races from mechanical mixtures even on what is generally considered to be a completely susceptible variety.

A number of physiologic races differed in characteristics other than pathogenicity. They include certain morphological variations in chlamydospores and smut balls and in color of the spore mass and certain differences in their effect on the host plants as regards stunting, dropping of the awns, and degree of laxness in the spikes.

Turkey (C. I. 6175) and Mindum (C. I. 5296) were found to differ in susceptibility to certain races of both species when grown under different environmental conditions. This variation appears to be due to the effect of environment on the host rather than on the fungus and is expressed only in the reaction of certain varieties to certain physiologic races.

There are several agronomically desirable wheat varieties that are highly resistant to a large number of the known physiologic races of *Tilletia tritici* and *T. levis*. No two of these varieties are susceptible to the same race. It should be possible, therefore, to obtain hybrids in which factors governing resistance to all races of *Tilletia* are combined with those governing other desirable agronomic characters.

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FUSARIUM RESISTANCE IN WISCONSIN ALL SEASONS CABBAGE [1]

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INTRODUCTION

The control of the yellows disease of cabbage (*Brassica oleracea* L.) has been effected through the development of varieties of cultivated cabbage (*B. oleracea capitata* L.) that are resistant to the soil-borne parasite, *Fusarium conglutinans* Wr. Wisconsin All Seasons, one of the first of the yellows-resistant varieties developed, was released for commercial distribution in 1920 (*6*).[3] The parent strain (XXV–7–2s) was a progeny derived from the self-pollination of a plant of the variety All Seasons which had remained free from disease on yellows-infested soil in the Wisconsin field trial of 1916. Trials of this progeny in the field in 1917 and 1918 gave 5 and 1 percent disease, respectively, while All Seasons showed 80 and 60 percent, respectively. Heads selected from this strain served as the foundation stock from which the resistant variety Wisconsin All Seasons was derived. This variety is widely used for sauerkraut manufacture in the Middle West and consistently shows a high percentage of disease-free individuals on the most severely infested field soils.

Recent studies with Wisconsin Hollander (*1*) have shown that it did not contain the single dominant gene for resistance to yellows, as has been described in a number of the resistant varieties of cultivated cabbage, in brussels sprouts (*Brassica oleracea gemmifera* DC.), in kohlrabi (*B. oleracea caulo-rapa* DC.), and in wild cabbage (*B. oleracea*) (*2*, *8*, *9*, *10*, *11*). Resistance to *Fusarium* in Wisconsin Hollander is apparently due to a number of factors, cumulative in effect, which control a type of resistance that is effective at moderate soil temperatures but not at constant soil temperatures of about 24° C. Resistance in the other investigated varieties of cabbage and allied forms is due to a single dominant factor and remains effective at soil temperatures of 24° and slightly above on heavily infested soils. This type of resistance is designated as type A. The other, manifested by Wisconsin Hollander, is designated as type B. The chief difference between the two types, on which the technique of distinguishing them is based, is that type A is due to a single dominant gene, whereas type B is more complex genetically. Plants of type A, when crossed with a very susceptible line, yield F_1 progenies that remain free from yellows when grown on infested soil at a constant

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[3] Reference is made by number (italic) to Literature Cited, p. 510.

temperature of 24°. Plants of type B so crossed yield progenies that succumb to the disease on infested soil at 24°.

Wisconsin All Seasons has never been examined critically as to the nature of its resistance. The studies herein reported were undertaken to determine the nature of resistance in this variety and to improve the variety in uniformity and in other characters.

MATERIALS AND METHODS

The cabbage material from which selections were made represented two strains of the resistant variety Wisconsin All Seasons (WAS–1 and WAS–2). Other lots used for comparative purposes included two lots of the susceptible variety All Seasons (AS–1 and AS–2), a highly susceptible commercial variety of Danish Ballhead, and a homozygous susceptible line (77– series) derived from a Flat Dutch variety. As the result of numerous field and greenhouse trials, the last two lots of material were known to be very highly susceptible to the yellows disease and apparently carried little or none of type B resistance. Individuals from the 77– line were used as a source of pollen for crosses. Throughout the discussion of results this line will be referred to as a highly susceptible line of cabbage.

The material used for breeding purposes was handled in the manner described by earlier writers (*11*). Controlled pollination was effected in the greenhouse, where flowering branches were covered with glassine bags to reduce the chance of contamination by other pollen. Field trials were conducted on thoroughly infested soil in Racine and Kenosha Counties, Wis. Seedbeds were planted on yellows-free soil, and the seedlings were transplanted to the trial plot during the early part of July. The development of disease symptoms was followed throughout the season, bamboo stakes being used as permanent markers of plants showing external symptoms of the yellows disease.

To supplement the field studies, additional trials were conducted on yellows-infested soil in the greenhouse. Artificially inoculated soil (prepared by adding corn meal-sand cultures of the yellows organism to yellows-free soil), naturally infested soil from Racine County, or a mixture of the two types of soil was used. Thorough infestation of the several soils was evident from the reaction of susceptible seedlings used in each greenhouse experiment. The trials were conducted under semicontrolled and controlled soil temperatures. In the first case, the seedlings were transplanted to benches or flats containing yellows-infested soil and a soil temperature favorable for disease development was attained by adjusting the surrounding air temperature of the house. Wide fluctuations in the soil temperature occurred, but in general a temperature favorable for the expression of disease symptoms was maintained. Controlled soil temperatures were secured by the use of Wisconsin soil-temperature tanks.

EXPERIMENTAL RESULTS

During the early progress of the work it was observed that in the field trials of the progenies from self-pollination there was a marked deficiency of diseased individuals in supposedly homozygous susceptible (*rr*) progenies and in segregating (*Rr*) progenies. It was evident that there exists in Wisconsin All Seasons a condition in some respects

similar to that observed in Wisconsin Hollander by Anderson (*1*) (type B resistance). The inheritance of type A resistance and of type B resistance is discussed separately in the following sections.

TYPE A RESISTANCE

F_1 Progenies

The initial head selections were made from two lots of the Wisconsin All Seasons variety in 1931 and 1932. The heads selected in 1931 represented plants of the best type from a population that had been subjected to severe selection for resistance in a yellows trial conducted in the greenhouse; some of those selected in 1932 were from a yellows-infested field, others from a yellows-free field. The seed grown from the heads selected in 1931 and 1932 was produced under conditions of controlled pollination in the greenhouse in 1932 and 1933, respectively. Some of the blossoms on each plant were self-pollinated and some were crossed with pollen from a highly susceptible line.

The F_1 progenies from self-pollination were tested in the field in 1933, and certain of the lines were retested in the field trials of 1934 and 1935. The progenies of crosses were tested almost entirely in the greenhouse, although several were included in the field trials.

The results of trials in the field, in the greenhouse under semicontrolled soil temperatures, and in the soil-temperature tanks are considered separately (table 1). On the basis of their disease reaction under controlled conditions, the F_1 progenies have been grouped into three classes according to the apparent genotype of the parent plant. The homozygous-susceptible (*rr*) class includes those plants the progenies of which comprise only susceptible individuals. The heterozygous-resistant class (*Rr*) includes those plants the progenies of which, from self-pollination, show approximately 25 percent of the individuals diseased and, from crossing with a homozygous-susceptible line, approximately 50 percent of the individuals diseased. The homozygous-resistant class (*RR*) of plants yielded no significant number of susceptible individuals in the F_1 progeny either from self-pollination or from crossing with a homozygous-susceptible line.

TABLE 1.—*Behavior of F_1 progenies of selected plants showing type A resistance to Fusarium in field and greenhouse*

Conditions of test	Genotype of parent plant	Progenies from self-pollination			Progenies from cross of selected plant with susceptible line		
		Progenies tested	Total plants	Diseased plants	Progenies tested	Total plants	Diseased plants
		Number	*Number*	*Percent*	*Number*	*Number*	*Percent*
Field	*rr*	11	440	27	3	142	54
	Rr	27	2,017	5			
	RR	9	460	.2			
Greenhouse, semicontrolled	*rr*	2	63	92	4	227	98
	Rr	20	2,715	19	34	3,445	46
	RR	2	181	0	9	624	0
Greenhouse, controlled (24° C.)	*rr*	7	218	91	8	643	98
	Rr	21	1,463	22	16	1,152	48
	RR	7	200	0	6	236	0

It soon became evident that the genotypes could not be distinguished on the basis of field trials alone. However, under the more favorable conditions for the disease in the greenhouse trials, both semicontrolled and controlled, the genotypic constitution of plants could be determined quite accurately on the basis of progeny tests.

F_2 Progenies

From 19 of the F_1 selfed progenies selections were made for seed production representing the three genotypes of type A resistance, and F_2 selfed seed was secured from 108 of the F_1 plants. From 103 of the plants seed was obtained from a cross with susceptible plants. The F_2 selfed progenies and the F_1 progenies from the susceptible crosses were tested in the field, some of them during two successive seasons, as well as in the greenhouse. The disease reaction in the greenhouse trials showed that they were derived from mother plants representing one or another of the three genotypes (table 2). Lack of expression of disease symptoms was very evident in certain of the progenies in the field trials, preventing an accurate determination of the number of individuals in the susceptible class. However, when such progenies were tested in the greenhouse this difficulty was largely overcome. Selections from F_1 selfed progenies which had been classified as homozygous for resistance were found to yield completely resistant F_2 families.

TABLE 2.—*Behavior of F_2 progenies showing type A resistance to Fusarium in field and greenhouse*

Conditions of test	Genotype of parent plant	Progenies from self-pollination [1]			Progenies from cross of selfed F_1 with susceptible line		
		Progenies tested	Total plants	Diseased plants	Progenies tested	Total plants	Diseased plants
		Number	*Number*	*Percent*	*Number*	*Number*	*Percent*
Field	*rr*	18	880	12	7	240	58
	Rr	50	2,641	5			
	RR	39	2,291	0			
Greenhouse, semicontrolled	*rr*	8	746	72	16	1,290	92
	Rr	42	3,128	22	46	2,243	45
	RR	26	1,512	.07	38	1,810	.2
Greenhouse, controlled (24° C.)	*rr*	13	830	89	11	475	99
	Rr	8	355	18	8	480	54
	RR	13	338	0	1	75	0

[1] Selfed 2 generations.

F_3 Progenies

From the selfed F_2 material in the field trials, selections were made chiefly from the homozygous-resistant lines. Inasmuch as resistance had been established in a homozygous form in many of the plant lines, the selections were secured from those lines that appeared to approach most nearly the ideal type of Wisconsin All Seasons. The plants were paired in the greenhouse, sib crosses were made, and on many of the plants a cross with a very susceptible line was also made. The sib-cross progenies were tested for yellows reaction in the field, and all remained free of disease symptoms with the exception of three of the progenies, in each of which one yellows plant was found. Further

study in the greenhouse of these progenies and of the corresponding susceptible-cross progenies revealed that one of the parents in each of the sib crosses was heterozygous for resistance, apparently due to a chance contamination in the F_2 generation with pollen of a susceptible line.

The results presented indicate that a certain percentage of individuals of the Wisconsin All Seasons variety carry the factor for type A resistance. However, it was pointed out that in the initial selections from this variety a large percentage of the individuals carrying only type B resistance were probably eliminated by exposure to high soil temperatures. A majority of the individuals surviving this treatment were either heterozygous or homozygous for the type A gene (*R*). However, certain of the progenies classed as homozygous recessive for this gene (*rr*) on the basis of their greenhouse reaction were highly resistant in the field. In the F_3 selfed progenies having this constitution, only 2 percent of the plants became diseased in the field as contrasted with 93 percent diseased at a soil temperature of 22° to 24° C. in the greenhouse. Resistance in these lines was comparable to that described by Anderson (*1*) in Wisconsin Hollander and is discussed as type B resistance in the following section.

TYPE B RESISTANCE

As pointed out by Anderson (*1*), the expression of type B resistance is most pronounced at the lower soil temperatures on infested soil in field and greenhouse. It was observed that the progenies in Wisconsin All Seasons that were heterozygous or homozygous recessive for type A resistance commonly showed a percentage of diseased individuals lower than the theoretical, particularly in field trials in which commercial susceptible varieties and very susceptible selections from a variety other than Wisconsin All Seasons consistently showed a high incidence of disease. Inasmuch as the presence of type A resistance in any progeny obscures the expression of type B resistance, the studies herein reported were confined to the progenies that were homozygous for the recessive gene, *r*.

In table 3 are presented results of field and greenhouse trials of lines of Wisconsin All Seasons in which type A resistance was lacking. In the field trials the progenies showed a range from all plants diseased (25–67 selfed and 25–117 selfed) to complete freedom from apparent symptoms. Several of the susceptible-cross progenies included in the field trials showed a lower degree of resistance than did the selfed progenies of the female parents of those crosses. When subjected to trials in the greenhouse the progenies gave a much closer approximation to the expected amount of disease than was obtained in the field trials, showing the important influence of soil temperature in the expression of type B resistance. In order to secure additional data concerning the effect of soil temperature on this type of resistance, experiments were conducted in the soil-temperature tanks over a range of soil temperatures. The expression of symptoms increased with rise in soil temperature, not only in percentage of plants diseased but also in the severity of disease manifestation. The influence of soil temperature upon the rate of disease development and severity of expression is illustrated graphically in figure 1.

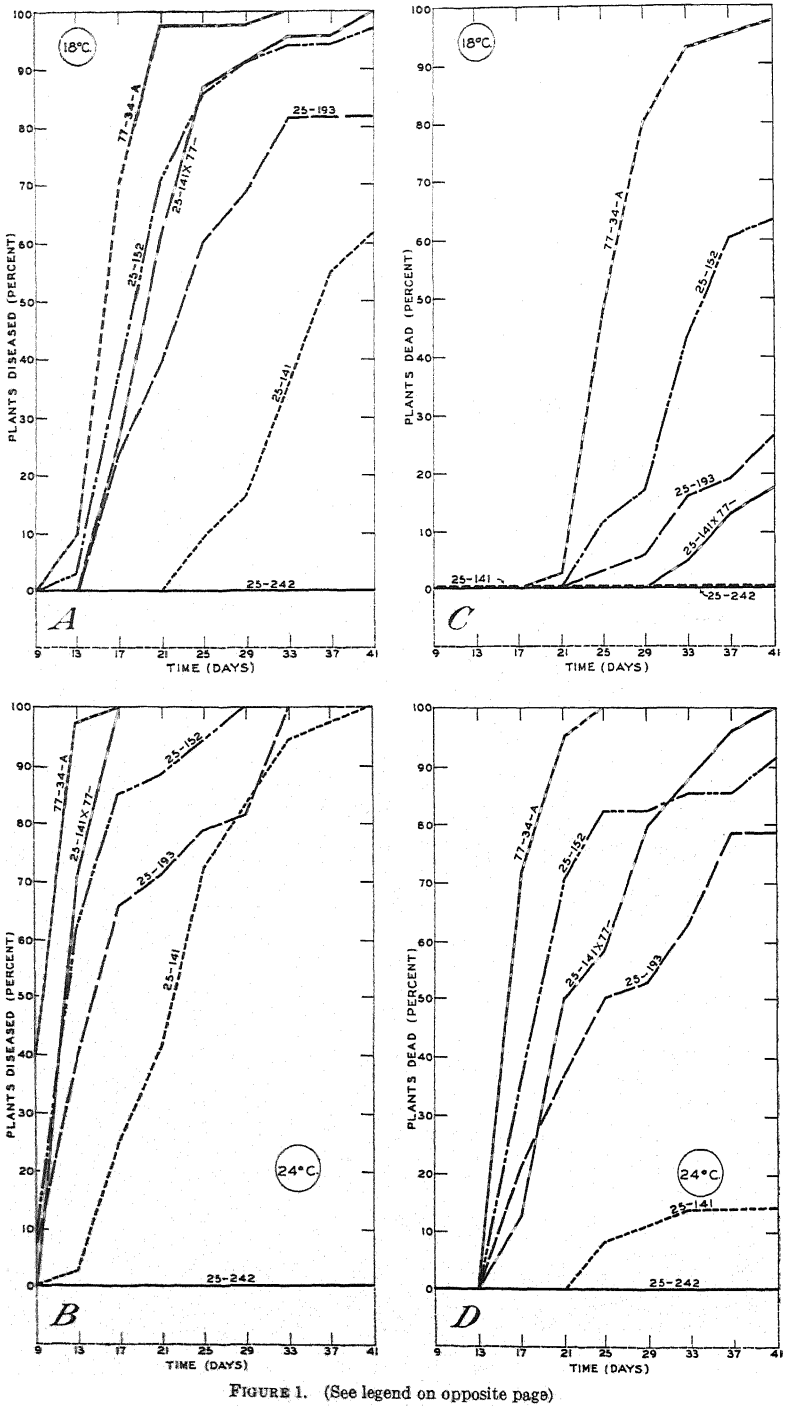


FIGURE 1. (See legend on opposite page)

TABLE 3.—*Occurrence of disease in F_1 progenies of Wisconsin All Seasons cabbage homozygous recessive for type A resistance to Fusarium but carrying various degrees of type B resistance*

Parent plant no.	Progenies from self-pollination of selected plants							Progenies from cross of selected plant with homozygous susceptible line						
	Field trial			Greenhouse, semicontrolled		Greenhouse, controlled (24° C.)		Field trial			Greenhouse, semicontrolled		Greenhouse, controlled (24° C.)	
	Year	Total plants	Diseased plants	Total plants	Diseased plants	Total plants	Diseased plants	Year	Total plants	Diseased plants	Total plants	Diseased plants	Total plants	Diseased plants
		Number	Percent	Number	Percent	Number	Percent		Number	Percent	Number	Percent	Number	Percent
25-28	1933	22	0			33	97	1934	19	0	97	86	126	97
	1934	2	0					1935	37	57				
25-46	1933	52	27			31	94	1934	34	76	20	85	200	99
	1934	9	11											
25-52	1933	9	78			12	100						49	100
25-53	1933	11	0										65	98
25-54	1933	52	65			73	100							
	1934	11	27											
25-58	1933	52	25										77	99
	1934	18	22											
	1935	12	25											
25-60	1933	56	14	26	96	48	67				63	87	33	97
25-67	1933	14	100											
25-68	1933	53	6	37	89			1934	28	32	47	94	69	97
	1934	27	0					1935	24	83				
	1935	7	0											
25-73	1933	12	8			11	91						24	96
25-79	1933	21	57			10	100							
25-117	1933	23	100											
25-136	1934	25	0			45	96				51	100	32	100
25-137	1934	25	0			35	86				35	100		
25-139	1934	17	0			5	80				33	100	59	97
25-141	1934	51	0	6	50	104	80				26	100	24	100
25-152	1934	46	22			114	95				126	90	29	100
	1935	30	80											
25-193	1934	65	18	223	80	185	89	1935	70	94	189	98	99	100
	1935	46	39											
25-205	1934	74	4	30	70	44	93	1935	46	70	124	91	45	98
	1935	30	27											
25-212	1934	44	7	124	62	62	98				139	87	24	100
25-218	1934	68	0	116	57						48	94		
	1935	39	0											

Progeny 25–242 selfed was homozygous for type A resistance and remained free of disease symptoms throughout the trial. Progeny 77–34–A was a very susceptible line, resulting from a cross between several plants that were used as the source of pollen in the susceptible crosses and that were homozygous susceptible for type A resistance. Progeny 77–34–A was practically free from type B resistance as indicated by the rapidity and severity of disease development. Selfed progenies 25–152, 25–193, and 25–141 were homozygous susceptible for type A resistance, but according to field trials (table 3), carried

FIGURE 1.—Comparison of four progenies (25–141 selfed, 25–141 × 77–, 25–152 selfed, and 25–193 selfed) of Wisconsin All Seasons cabbage, showing type B resistance to yellows, with a very susceptible line (77–34–A) and with a progeny (25–242 selfed) homozygous for type A resistance, grown on *Fusarium*-infested soil at constant temperatures of 18° and 24° C. *A* and *B*, Incidence of disease (total affected plants) at 18° and 24°, respectively. The rate of development of symptoms is most rapid at the higher soil temperature. *C* and *D*, Severity of disease (plants dead) at 18° and 24°, respectively. At the lower soil temperature the very susceptible line (77–34–A) succumbed promptly, whereas the progenies carrying type B resistance varied in the rate and severity of disease development. At 24° these progenies succumbed, though less rapidly than did the very susceptible line. The hybrid progeny (25–141 × 77–) between plants high and low in degree of type B resistance was intermediate in its disease reaction between that of the parents. Progeny 25–242 selfed was homozygous for type A resistance and remained free of disease symptoms throughout the duration of the experiment.

different degrees of type B resistance. At both 18° and 24° C., these lines showed more resistance than did 77–34–A and maintained the same relative position as to degree of resistance that they had in the field trials. At 18°, 25–141 selfed showed but 61 percent of the plants diseased and none dead, whereas at 24° all plants were diseased and 14 percent were dead at the conclusion of the experiment. The susceptible-cross progeny of plant 25–141 gave a disease reaction intermediate between that of 25–141 selfed and 77–34–A, substantiating the evidence that, to some extent, type B resistance was transmitted to



FIGURE 2.—Comparison of three lots of cabbage showing varying degrees of type B resistance; trial on naturally infested soil in Kenosha County, Wis., in 1935. *A*, Progeny 25–425s, F_2 inbred generation, remained free of apparent symptoms. *B*, Progeny 25–462 × 77–, a cross of an F_2 plant with a very susceptible line, showed 17 plants diseased in a population of 50; of the diseased plants, 16 were only slightly affected and 1 was dead. *C*, A commercial susceptible line of the Danish Ballhead type; practically all of the plants were diseased, with the majority either severely diseased or dead. The same progenies at a constant soil temperature in the greenhouse are shown in figure 3.

and expressed in the F_1 hybrid of a cross between lines high and low in expression of this character.

From certain of the lines with type B resistance, individuals were selected in the 1933 field trials that had failed to show symptoms. The F_2 inbreds remained, with one exception, entirely free from symptoms in the field (fig. 2), and displayed a degree of type B resistance equal to or exceeding that of their parental lines (table 4). This result was anticipated, inasmuch as the F_2 inbreds were secured from F_1 plants that had remained free from disease in the field and that presumably had carried more type B resistance than did sister plants that had manifested symptoms. In the field, progenies from the crosses with susceptibles showed disease ranging from 5 to 73 percent. The behavior in the field of the F_2 progenies from self-pollination and the progenies of susceptible crosses indicated that type B resistance was dispersed to some extent when a cross was made with plants carrying little or none of this resistance. In the greenhouse the resistance of the F_2 inbreds was broken down (fig. 3).

TABLE 4.—*Occurrence of yellows in F_2 and F_3 progenies selected from plants free from type A resistance to Fusarium but exhibiting type B resistance*

Parent progeny no.	Generation and progeny no.	Progeny from self-pollination							Progeny from cross of inbred F_1 or F_2 with a homozygous susceptible line						
		Field trial			Greenhouse, semicontrolled		Greenhouse, controlled (24° C.)		Field trial			Greenhouse, semicontrolled		Greenhouse, controlled (24° C.)	
		Year	Total plants	Diseased plants	Total plants	Diseased plants	Total plants	Diseased plants	Year	Total plants	Diseased plants	Total plants	Diseased plants	Total plants	Diseased plants
	F_2		*No.*	*Pct.*	*No.*	*Pct.*	*No.*	*Pct.*		*No.*	*Pct.*	*No.*	*Pct.*	*No.*	*Pct.*
25-58s	25-262	1934	57	0	10	80	48	56	1935	26	73	37	100	43	100
		1935	19	0											
	25-265	1934	16	0			36	86				72	75	29	100
		1935	2	0											
25-68s	25-275	1934	53	0					1935	26	23	37	86		
		1935	38	0											
	25-423	1935	26	0	99	79	43	94	1935	26	31	158	96		
	25-424	1935	14	0					1935	25	24	127	88	50	100
	25-425	1935	36	0	138	74	61	95							
25-46s	25-411	1935	22	9			48	100	1935	21	5	55	95	41	100
	25-412	1935	14	0								33	73		
	F_3														
25-262s	25-461				158	88	85	87				53	96		
	25-462	1935	74	3	95	84	60	93	1935	50	34	175	80	59	86
25-265s	25-465	1935	4	0											
25-275s	25-468	1935	16	0								10	80		
	25-472	1935	19	0											

Several of the F_2 progenies were carried through another generation. Although the number of progenies involved was small, there was evidence that type B resistance was relatively stable through three generations of self-pollination (fig. 4). As usual in greenhouse trials, type B resistance was broken down upon exposure of seedlings to higher soil temperatures.

TABLE 5.—*Occurrence of disease in F_2 progenies of a cross between plants carrying type B resistance and a very susceptible plant*

Cross no.	F_1 progeny no.	F_2 progenies from self-pollination							Progeny from backcross with homozygous susceptible line						
		Field trial			Greenhouse, semicontrolled		Greenhouse, controlled (24° C.)		Field trial			Greenhouse, semicontrolled		Greenhouse, controlled (24° C.)	
		Year	Total plants	Diseased plants	Total plants	Diseased plants	Total plants	Diseased plants	Year	Total plants	Diseased plants	Total plants	Diseased plants	Total plants	Diseased plants
			No.	*Pct.*	*No.*	*Pct.*	*No.*	*Pct.*		*No.*	*Pct.*	*No.*	*Pct.*	*No.*	*Pct.*
25-28 × 77	25-401	1935	26	54											
	25-402	1935	30	37											
	25-403	1935	26	73			45	100	1935	38	87			45	100
	25-404	1935	30	33			35	100	1935	28	93				
	25-405	1935	25	8			27	89							
25-46 × 77	25-415	1935	43	95	107	100	47	100				173	100	49	100
	25-417	1935	34	88	42	100	52	100	1935	54	61	169	93	49	100
	25-418	1935	41	49	71	97	55	98	1935	41	39	73	99	54	100
	25-419	1935	25	72			7	100				150	97		
	25-420	1935	33	64								100	99		
25-68 × 77	25-426	1935	34	71	101	99	62	100	1935	27	93	180	99	50	100
	25-429	1935	38	82	56	100	60	100	1935	41	100	92	97	56	100

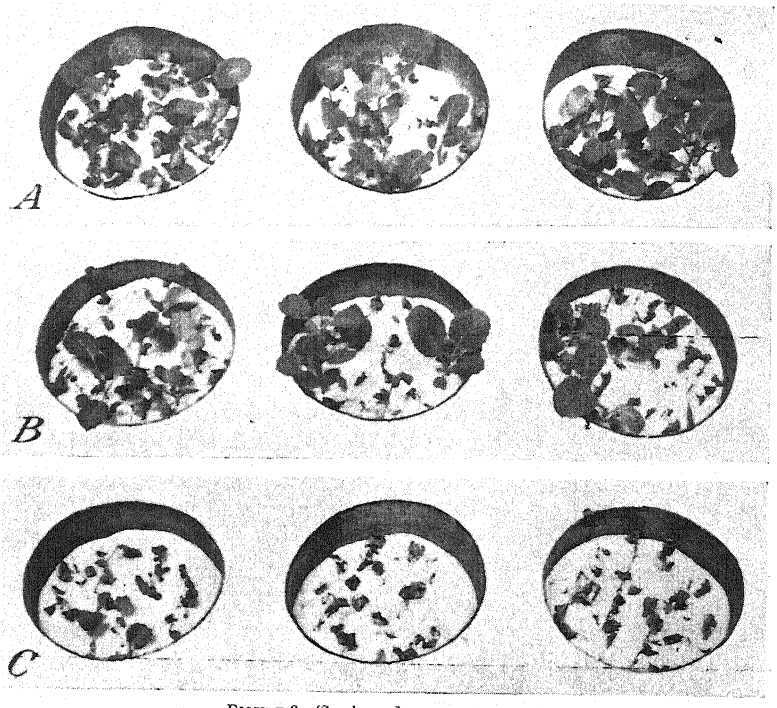


FIGURE 3 (See legend on opposite page)



FIGURE 4 (See legend on opposite page)

From the progenies of crosses of plants 25–28, 25–46, and 25–68 with susceptibles, selections were made of those progenies that showed in the 1934 field trial 0, 76, and 32 percent plants diseased, respectively (table 3). Seed was produced from the selected heads, and the results of the resistance tests are presented in table 5.

DISCUSSION

The object of this investigation was to determine the nature and inheritance of resistance to *Fusarium* in the Wisconsin All Seasons variety of cabbage. The presence of type A resistance, due to a single dominant gene, was demonstrated in inbred progenies from many of the plant selections. This was in accord with the results of other workers on other varieties of cabbage and certain of the subspecies of *Brassica oleracea*. However, it was found that in Wisconsin All Seasons there was also a second type of resistance, referred to as type B. The latter type is apparently similar to that described by Anderson for Wisconsin Hollander, in which type A resistance is lacking. Type B resistance is characterized by an optimum manifestation at the lower soil temperatures, but at the higher soil temperatures it breaks down and the plants succumb to the disease. This response to soil temperatures made it impossible to differentiate accurately between the two types of resistance in field trials and necessitated trials under conditions of higher soil temperatures in the greenhouse.

Inasmuch as the presence of type A resistance, either in the homozygous dominant (*RR*) or heterozygous (*Rr*) form, obscured the manifestation of the presence of type B resistance, it was necessary to work with progenies that were homozygous recessive (*rr*) for type A resistance. Such progenies varied greatly in the degree of type B resistance, showing a range in the 1933 field trials from all plants diseased in two of the progenies to complete freedom from symptoms in other progenies. Selections from F_1 progenies, which apparently carried a considerable degree of type B resistance, yielded F_2 and F_3 progenies that maintained a high degree of such resistance in succeeding field trials. Nevertheless, the resistance of all was capable of being broken down under greenhouse trials, where more severe conditions prevailed. It was observed, however, that the progenies tested in the greenhouse displayed the same relative degree of type B resistance as was shown in the field trials. The presence of type B resistance was manifested in greenhouse trials by the delayed development of symptoms and their less serious expression. In some of the progenies showing a high degree of type B resistance it was only by prolonged exposure at the higher soil temperatures that a complete or nearly complete break-down of resistance could be secured.

FIGURE 3.—Comparison of the three lots of cabbage shown in field trials in figure 2, when grown on infested soil in the greenhouse at a controlled soil temperature of 23° C.; photographed 19 days after being transplanted. *A*, Progeny 25–425s: 77 percent diseased and 28 percent dead. *B*, Progeny 25–462 × 77–, a susceptible cross of an F_2 plant: 79 percent diseased and 63 percent dead. *C*, A commercial susceptible line of the Danish Ballhead type: 100 percent diseased and 88 percent dead. The relative resistance shown in the field trials was maintained; but the infection conditions were more severe, and all plants finally succumbed to the disease.

FIGURE 4.—Comparison of two lines of cabbage, both homozygous for the recessive gene (*r*) for type A resistance but differing in the degree of type B resistance. *D*, Line 77–, in which there is little or no type B resistance. *A*, *B*, and *C*, The F_1, F_2, and F_3 progenies (25–58, 25–262, and 25–462), respectively, of a line in which a high degree of type B resistance occurred. In the field trials there were 25, 0, and 3 percent plants diseased in the F_1, F_2, and F_3 progenies, respectively, but these progenies showed close to 100 percent of plants susceptible at a constant soil temperature of 24° C. (tables 3 and 4).

In lines of cabbage carrying both type A and type B resistance, the latter type is distinguishable only in those individuals that may segregate as homozygous recessives for type A resistance. That type B resistance is of importance is evident in the behavior of the resistant variety Wisconsin All Seasons. On thoroughly infested soil in Wisconsin this variety seldom shows as much as 10 percent disease; whereas, when tested at high soil temperatures (24° C.) in the greenhouse, it may show as high as 30 percent disease. Under field conditions there are apparently a considerable number of homozygous recessives for type A resistance that contain a sufficiently high degree of type B resistance to enable them to remain free of apparent disease symptoms. Wisconsin Hollander, a variety of cabbage that carries only type B resistance, illustrates the effective value of the latter in seasons when temperatures are moderate. Conversely the ineffectiveness of type B resistance is very evident in Wisconsin Hollander during seasons of high soil temperatures, when a major part of the crop often becomes diseased.

Evidence of the presence of what we now refer to as type B resistance may be had by an examination of earlier studies on inheritance of resistance to *Fusarium* in cultivated cabbage and in other subspecies of *Brassica oleracea*. Walker (*8*) earlier pointed out that differences in the rate and severity of disease development occurred among various susceptible progenies in certain cabbage varieties and that in addition to the main gene for resistance there might be hereditary factors which would modify the expression of the disease. In the light of present knowledge it is reasonable to believe that many of the plant lines which displayed a considerable number of plants in the "mildly susceptible" or "recovered" classes were expressing type B resistance.

The extent to which type B resistance can be concentrated in any given line of cabbage is problematic. The results of present studies indicate that a high degree of type B resistance was maintained through three generations by self-pollination.

The inheritance of resistance to other vascular Fusaria has been studied in the pea (*Pisum sativum* L.), in flax (*Linum usitatissimum* L.), and in cotton (*Gossypium* spp.). Wade (*7*) working with a number of varieties of canning peas, found that resistance to fusarium wilt (*Fusarium orthoceras* var. *pisi* Linford) was based on a single dominant gene. There is very little suggestion in his results of a condition similar to that due to type B resistance in cabbage. As a result of studies on the inheritance of resistance in flax to wilt (*F. lini* Bolley) Burnham (*3, pp. 738, 748*) said:

> The fact that there are strains that apparently breed true for different degrees of wilting may be taken as evidence that several factors are concerned in wilt resistance. One might assume different genetic complexes for the various degrees of resistance; and, further, that each has its own normal curve of variation in development of the resistant property. Under different environments different proportions of the least resistant side of the curve would succumb. The occurrence of some wilt in resistant lines which probably are homozygous, makes genetic analysis of the crosses more difficult. * * * Crosses between certain resistant lines of different origin showed a high percentage of wilt, indicating that they may carry different factors for resistance.

Burnham (*3*) found in general that flax families showing high or low percentages of wilt in the field gave the same reaction in the greenhouse, which is similar to the behavior of cabbage lines with type B resistance.

Studying the inheritance of resistance to fusarium wilt (*Fusarium vasinfectum* var. *aegyptiacum* Fahmy) in a number of varieties of Egyptian cotton, Fahmy (*5*) concluded that resistance and susceptibility are hereditary characters, immunity being dominant and appearing to give a simple Mendelian segregation. In crosses between immune plants and highly susceptible ones, he found in the F_2 generation three immune to one susceptible when plants that showed mild symptoms and recovered, as well as plants that succumbed, were considered as susceptible. The class that Fahmy designated as resistant included plants which showed typical leaf mottling in the seedling stage but which recovered and developed into apparently normal plants. In the adult stage such plants commonly showed a restricted darkening of the vascular cylinder of the root, from which the causal organism of wilt could be isolated. Differences were noted by Fahmy in the rate of development of symptoms in the different lines studied. It is probable that in the material studied by him there were modifying factors for resistance, similar to type B resistance in Wisconsin All Seasons cabbage, as well as resistance controlled by a single dominant gene.

Edgerton and Moreland (*4*) made no attempt to determine the inheritance of resistance to *Fusarium* in the tomato, but their results indicate that in the material they observed resistance is probably of the intermediate type, similar to type B resistance in cabbage. It is of significance to note that, while the percentage of plants showing evident (external) symptoms of wilt in the wilt-resistant tomato varieties was rather low, the actual percentage of wilt-infected plants, as determined by the cutting of stems, was much higher. A similar condition has been noted in cabbage plants of susceptible progenies which may manifest no apparent symptoms throughout the growing season but which, on examination of cut stems or roots, may show evidence of infection.

SUMMARY

This paper reports a study of two types of resistance of the Wisconsin All Seasons variety of cabbage to the yellows organism, *Fusarium conglutinans* Wr. Plants were selected from a population that had undergone a severe reduction in numbers from yellows as well as from a population grown on yellows-free soil. The plants were self-pollinated and crossed with homozygous-susceptible plants from a highly susceptible line.

The progenies were tested for yellows reaction on thoroughly infested soil in the field and in the greenhouse. The greenhouse trials were conducted with semicontrol and control of soil temperatures.

The Wisconsin All Seasons variety carries at least two types of resistance. Type A resistance, due to a single dominant gene, is apparently similar to that described for other varieties of cabbage, for brussels sprouts, and for kohlrabi. It remains effective at relatively high soil temperatures (about 24° C.). Type A resistance was demonstrated in progenies of many of the plant selections. It was transmitted through the three generations of material studied. A second type of resistance, referred to as type B, was also found in Wisconsin All Seasons. It is apparently similar to that described for Wisconsin Hollander, and is complex genetically, apparently influenced by several genetic factors. It is characterized by having its most effective expression at the lower soil temperatures, while at the

higher soil temperatures of about 24° plants that have only type B resistance succumb to the disease.

Under the conditions prevailing in the field trials, type B resistance was effective in different degrees in the several progenies. Some, evidently carrying high degrees of such resistance, showed no external symptoms of the disease; other progenies gave evidence of less resistance by development of a considerable percentage of mild infection; and still others succumbed promptly to the disease.

In the greenhouse trials with controlled soil temperatures the expression of type B resistance was suppressed, all progenies eventually succumbing to the disease. Under semicontrolled or fluctuating temperatures, type B resistance was partially expressed, resulting in a delayed and less severe development of symptoms of the disease.

In the greenhouse trials, as in the field, different progenies showed differences in rate and severity of disease development, indicating various degrees of type B resistance.

The severity of yellows disease in Wisconsin All Seasons cabbage depends on the genetic make-up with reference to type A and to type B resistance, on soil temperature, and possibly on other conditions that influence the expression of resistance.

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FACTORS AFFECTING CHLOROSIS IN IRRIGATED WHEAT [1]

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INTRODUCTION

A tendency of Marquis wheat to turn pale when irrigated at germination has been noticed in previous studies, and a similar tendency has been observed in the field when heavy rains occurred soon after the wheat emerged. The pale-green color may last for 3 to 6 weeks before the plants recover their normal green appearance. This mild chlorotic condition has been attributed to the use of cold water for irrigation, but experimental data to support this belief are lacking. Because of this a study was made of the effect on chlorosis of (1) the temperature of the irrigation water at germination and (2) the type of fertilizer used.

EFFECT OF TEMPERATURE OF IRRIGATION WATER ON YIELD OF WHEAT IRRIGATED AT GERMINATION

EXPERIMENTAL METHODS

Studies on the effect of applying irrigation water of different temperatures, to Marquis wheat (*Triticum aestivum* L.) on Fort Collins loam (*7*) [2] were conducted for a 4-year period from 1927 to 1930, inclusive.

The experiment was carried on each year on land which had been summer-fallowed the previous year. This summer fallow was necessary in order to obtain a more uniform moisture content in the soil and also to eliminate volunteer grain and control weeds. In 1928 and 1929, a single series of 10 plots, each one five-hundredths of an acre in size, was used. In 1930, two series of similar plots were employed. Each series was planted to Marquis wheat at the rate of 90 pounds per acre. One drill width of 16 rows was planted in each series.

The wheat was planted on April 8 in 1929 and April 7 in 1930. Canvas covers similar to those described by Robertson et al. (*5*) were used to eliminate the effects of rainfall in 1929 and 1930. Water was applied at the rate of 6 acre-inches [3] to each plot at germination (*5*). The temperatures of the irrigation water applied in 1928 were 41° and 62° F., and in 1929 and 1930 they were 40° and 60°.

The basin method of irrigation was used throughout the investigation. The plots were diked and sufficient water was applied to give the required depth. The amount of water required for a given depth was measured for each plot. The temperatures were controlled by ice or steam, depending on whether the water in the pipe line was higher or lower than the temperature required. The source of the irrigation water is discussed in a previous publication (*5*).

[1] Received for publication Apr. 1, 1937; issued October 1937.
[2] Reference is made by number (italic) to Literature Cited, p. 520.
[3] Inches of irrigation water refer to inches in depth over the area.

The area of each plot harvested was one eight-hundred-and-seventy-firsts of an acre. The 10 center rows were harvested after 3 border rows on each side and 1 foot from each end had been discarded to eliminate possible border effect. The grain was cut 1 inch above the ground with lawn shears, tied in a sheaf, and carefully wrapped in cloth to protect the heads, leafy material, and straw. These sheaves were shocked under cover and allowed to cure for 3 weeks or more. The sheaves were then weighed and the grain threshed. The difference between the cleaned grain weight and the total grain and straw weight was used as the straw yield.

EXPERIMENTAL RESULTS

The studies conducted with irrigation water at temperatures of 40° and 60° F. applied at germination, show a slight difference in grain yield in favor of the plots receiving the water at 40°. However, in 1930 when the number of replications was sufficient to make the application of statistical methods possible, no significant differences were obtained. The straw yield was slightly higher for the plots irrigated with water at 60°, but was not significantly different (table 1).

TABLE 1.—*Yields of grain and of straw from plots irrigated at germination with water at 40° or 60° F.*

Year grown	Plots	Yield per plot when irrigation water at indicated temperature was used			
		40° F.		60° F.	
		Grain	Straw	Grain	Straw
	Number	*Grams*	*Grams*	*Grams*	*Grams*
1929	2	398	820	373	837
1930	4	330±12	617±26	318±12	656±26

Standard error obtained by the analysis of variance (*2*).

An examination of the soil-temperature curves in figure 1, *A* (1928), shows that there was a rise in temperature, in the first foot of soil, of about 4° F. 24 hours after treatment. The second day after irrigation, the temperature dropped to within 1.5° of the low-temperature treatment. After the fourth day the difference between the two treatments did not exceed 2°.

In the second foot (fig. 1, *B*, (1928) there was a difference of 5.5° F. between the two treatments 24 hours after the water was applied. The temperature had become equal after 3 days and fluctuated only slightly thereafter.

In 1929 (fig. 2, *A*,) the temperatures of the soil in the plots came within 0.5° F. of each other 3 days after treatment, and did not fluctuate beyond 1.5° during the 10-day period after irrigation. In the second foot (fig. 2, *B*), there was very little difference.

These results, considered in connection with the yield data, indicate that a difference in temperature in the irrigation water of 20° F., i. e., between 40° and 60°, has no effect on the yield of Marquis wheat irrigated at germination.

The minimum temperature of the air is plotted in figures 1 and 2, along with the soil temperatures. It will be noted that the soil

temperature seems to be influenced more by the fluctuation of the air temperature than by the temperature of the irrigation water. This is especially noticeable in the curves for temperature 4 days after the application of the irrigation water. The following theoretical discussion indicates that a rise in temperature of about 10° F. should be the maximum difference expected if outside influences have no effect on the soil complex and the water added is the only contributing factor to the temperature change.

If water and soil of different temperatures are mixed, the final temperature of the complex (assuming no energy changes due to chemical reactions) may be expressed by the equation:

$$\frac{c_1 m_1}{c_2 m_2} = \frac{^\circ F_2 - X}{X - ^\circ F_1} \qquad (1)$$

where c_1=specific heat[4], m_1=the mass, and $^\circ F_1$=the temperature of the constituent having the lower temperature, and c_2, m_2, and $^\circ F_2$=the corresponding properties of the constituent of higher temperature, and X=the final temperature.

If water is already present as soil moisture before irrigation, it might be expected that the heat capacity of the soil plus moisture would determine the final temperature. However, when water is added to the soil surface it does not mix intimately with the soil moisture already present, but to a large extent replaces it as the portion already present moves downward. It is a question as to how much exchange of heat there is between moisture in the soil and moisture added under these conditions. Furthermore, the upper layers of soil are changed a little by each succeeding increment of water which enters, so that at the completion of irrigation the surface will approach the temperature of the water added and the extreme depth of penetration will approach the temperature before irrigation. The problem is thus complicated by an unknown degree of mixing with the soil moisture and the development of a temperature gradient. Other complications, of course, are the effects of evaporation and heat radiation during the process.

If we assume that the water added warms or cools the soil only and not the moisture already present and that the evaporation and radiation effects may be neglected, the following example shows the average final temperature and depth of temperature change which should be expected from adding 6 inches of water at 60° F. to a soil at 40°:

Average field capacity of upper 2 feet (estimated)	18.0 percent.
Ratio of weight of soil to weight of water	5.55/1.

Substituting in equation 1—

$$\frac{0.2 \cdot 5.55}{1} = \frac{60 - X}{X - 40} = 49.47^\circ \text{ F.}$$

Weight of water applied per square foot of surface	31.25 pounds.
Average weight per cubic foot of soil (determined)	88.3 pounds.
Weight of water absorbed per cubic foot (18 percent of soil)	15.89 pounds.
Depth of water penetration and of temperature change (no. 3÷ no. 5)	1.96 feet.

[4] Approximate specific heat of soil obtained from Patten (4).

The result is approximately a maximum average rise of 10° F. in soil temperature to a depth of 1.96 feet. The graphs show, as could be expected, that the actual rise in the field was much less than this.

The change in temperature, while appreciable, is small compared with the change which could be expected from a snowstorm. Since the heat of fusion of ice is 79.6 calories, an inch of water as ice would cool an equivalent amount of water nearly 144° F.

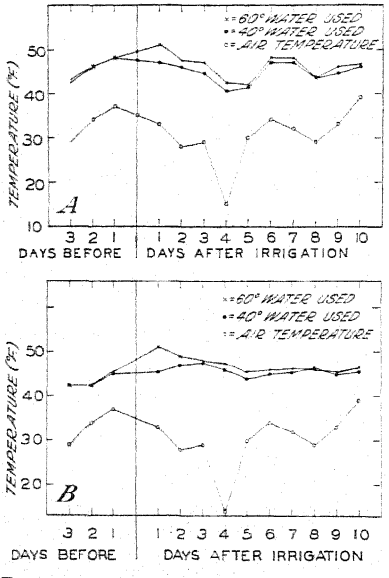


FIGURE 1.—Temperature of the first (*A*) and second (*B*) foot of soil for a 3-day period immediately before and a 10-day period immediately after irrigation with water at 40° and at 60° F., as compared with the air temperature, 1928.

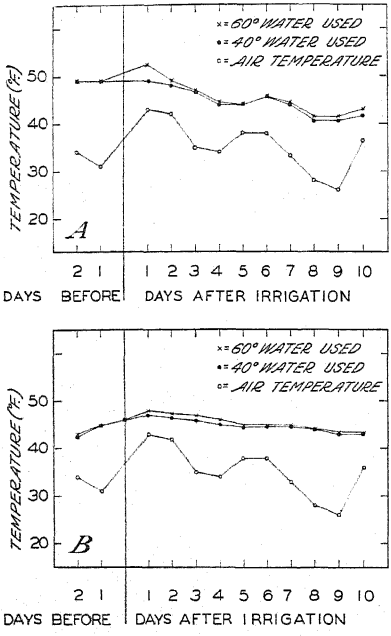


FIGURE 2.—Temperature of the first (*A*) and second (*B*) foot of soil for a 2-day period immediately before and a 10-day period immediately after irrigation with water at 40° and at 60° F., as compared with the air temperature, 1929.

EFFECT OF DIFFERENT FERTILIZER TREATMENTS AT GERMINATION ON THE YIELD OF WHEAT AND ON THE NITROGEN CONTENT OF SOIL, GRAIN, AND STRAW

EXPERIMENTAL METHODS

In 1933 a study was started to determine the effect of different fertilizer treatments at germination on the development of chlorosis and the recovery of Marquis wheat showing chlorosis. Uncovered plots of similar size to those used in the temperature study were employed. The plots were laid out in a 7 by 7 Latin square. The chemical treatments and the amounts used are given in table 2.

TABLE 2.—*Chemical treatments and amounts applied per acre*

Treatment	Salt used	Quantity per acre
		Pounds
Manganese sulphate	$MnSO_4+4H_2O$	318.70
Iron sulphate	$FeSO_4+7H_2O$	297.22
Superphosphate	$CaH_4(PO_4)_2+H_2O$	360.25
Ammonium-acid phosphate	$(NH_4)H_2PO_4$	328.80
Ammonium sulphate	$(NH_4)_2SO_4$	188.77
Calcium nitrate	$Ca(NO_3)_2+4H_2O$	337.35

Marquis wheat was planted on April 3, 1933, April 9, 1934, and April 1, 1935.

Treatment of plots.—In 1933 irrigation water was applied at the rate of 6 inches when the wheat had germinated and was just emerging. The various series were irrigated as follows: April 20, series E; April 24, series F and G; April 25, series H, I and plots 3, 4, 5, and 6 on Series J; April 26, series K and plots 7, 8, and 9 on series J. The chemicals were dissolved in water and sprinkled on the plots within 24 hours after the 6-inch irrigation. After treatment with the chemical, 1 inch of water was applied to each plot.

In 1934 a 6-inch irrigation was applied on April 20 to series A and B; on April 21 to series C; on April 23 to series D and E; and on April 24 to series F and G. The treatments were applied in a manner similar to that used in 1933. On June 20 and 21, an additional 3 inches was applied to each of the plots.

In 1935 a 4-inch irrigation was applied on April 5 to series A, B, and C. A similar irrigation was applied on April 6 to series D, E, and F. On April 8, series G was irrigated with 4 inches of water. This irrigation was necessary because of the dry season of 1934 and of the months of January, February, and March in 1935. On April 20, when the wheat had germinated, an additional 5-inch irrigation was applied to series A and B. On April 22, series C and D were irrigated. Series E, F, and G were irrigated on April 23. The chemicals were applied on all plots in series A to F on April 23 and to series G on April 24. Sufficient rain fell on April 24 and 25 to carry the fertilizers into the soil so no additional irrigation water was applied.

Statistical analysis.—In the interpretation of the data from the crop-and-soils tests, the analysis of variance *(2)* was used. The data were so arranged that the various interactions could be tested. Table 3 presents the analysis of variance for the yield of grain in grams per plot.

TABLE 3.—*Analysis of variance for yield of grain per plot for the 3-year period, 1933 to 1935, inclusive*

Variance due to—	Degrees of freedom	Sum of squares	Mean square	σ	F[1] *(6)*
Years	2	763,069	381,535	.....	180.65
Rows and columns within years	36	409,889	11,386	.....	5.39
Treatments	6	224,423	37,404	.....	17.71
Treatments x years	12	122,180	10,182	.....	4.82
Error	90	190,127	2,112	45.96	.....
Total	146	1,709,688	.....	.....	.....

[1] All higher than 1-percent point.

The F test indicates that there is a significant difference in the yields between years, rows, and columns within years, and treatments × years; the last-named difference shows that the treatments reacted differently in different years.

The test further shows that response of Marquis wheat to some of the fertilizer treatments is significant. Similar results were obtained for the straw yields and for the total yield of straw and grain combined.

In 1933 the effect of the various treatments first became noticeable about May 30. The plots treated with calcium nitrate were a much darker green than the other plots. The plots receiving ammonium fertilizer were a shade lighter, but could be distinguished from the other fertilizer and the no-treatment plots, which were light green in color. In 1934 and 1935, a similar condition was noticeable.

EXPERIMENTAL RESULTS

Yield of Grain and Straw

The average yields in grams per plot for the 3-year period are given in table 4.

TABLE 4.—*The 3-year average yield of grain and of straw from plots irrigated at germination and later treated with different fertilizers*

Treatment	Yield per plot			Treatment	Yield per plot		
	Grain	Straw	Total		Grain	Straw	Total
	Grams	*Grams*	*Grams*		*Grams*	*Grams*	*Grams*
$FeSO_4$	832	1,179	2,011	$(NH_4)_2SO_4$	886	1,319	2,205
$CaH_4(PO_4)_2$	838	1,189	2,027	$NH_4H_2PO_4$	911	1,349	2,260
$MnSO_4$	840	1,191	2,031	$Ca(NO_3)_2$	939	1,492	2,431
No treatment	844	1,202	2,046				
				Level of significance (2 S.E. (difference)	28	49	77

The grain yields of the plots treated with ammonium sulphate, ammonium phosphate, and calcium nitrate are significantly higher than the yields of the no-treatment plots. The yields of plots treated with iron sulphate, superphosphate, or manganese sulphate do not differ significantly from the check. The straw yields show the same trend. The ammonium- and nitrate-treated plots gave the highest yields. These data indicate that the chlorotic condition of Marquis wheat produced by early irrigations evidently is due to a nitrogen deficiency.

To study further the effect of early irrigations on Marquis wheat, soil samples were taken at intervals throughout the season and analyzed for nitrate nitrogen. Samples were taken in all plots, making, in all, seven samples for each treatment. The samples were taken for the following depths of soil: 0–6, 6–12, 12–24, and 24–36 inches. The nitrate nitrogen was determined by the method described by Gardner and Robertson (*3*), p. 5.

Nitrate Nitrogen Content of Soil

Soil samples were taken in all plots after the irrigation and chemical treatments had been applied. The samples were taken on the following dates: April 29, 1933; April 27, 1934; and May 1, 1935. Different

composite samples were drawn for the different tests. The nitrate nitrogen is recorded in parts per million as nitrogen. Table 5 gives the average nitrogen content of the soil for different depths taken in the spring of 1933, 1934, and 1935.

TABLE 5.—*Average quantity of nitrate nitrogen recorded as nitrogen in parts per million for the different depths of soil taken in the spring after irrigation and chemical treatment were applied and in August after the wheat had been harvested on the variously treated plots for the 3-year period 1933 to 1935, inclusive*

SPRING

Treatment	Nitride nitrogen at soil depths indicated (in inches)				
	0–6	6–12	12–24	24–36 [1]	0–36 [1]
	P. p. m.	*P. p. m.*	*P. p. m.*	*P. p. m.*	*P. p. m.*
$Ca(NO_3)_2$	9.0	5.0	7.9	12.6	11.2
$(NH_4)_2SO_4$	4.4	4.3	5.1	9.9	8.3
$(NH_4)H_2PO_4$	4.2	3.8	4.4	9.2	7.3
$MnSO_4$	3.2	2.9	4.9	10.3	8.0
$FeSO_4$	2.7	3.6	5.0	9.5	7.1
No treatment	2.6	2.8	5.2	11.3	8.0
$CaH_4(PO_4)_2$	2.5	2.9	6.1	11.2	8.4
Level of significance	[2] 1.3	1.7	1.7	2.8	1.9

AUGUST

$Ca(NO_3)_2$	3.0	1.6	1.9	4.9	2.7
$(NH_4)_2SO_4$	2.9	1.5	2.6	3.5	2.4
$(NH_4)H_2PO_4$	3.1	1.8	1.7	3.2	2.4
$MnSO_4$	2.8	1.6	1.9	3.0	2.0
$FeSO_4$	2.9	1.6	2.0	2.9	2.0
No treatment	3.0	1.5	1.8	1.9	1.7
$CaH_4(PO_4)_2$	3.0	1.4	1.9	2.4	1.9
Level of significance	.55	.53	.54	2.1	.8

[1] 2-year average 1933 and 1935.

[2] Each depth was set up as a separate experiment and the standard error obtained by the analysis of variance. A test for homogeneity was applied to a complex experimental set-up for all of the data for nitrogen in the 4 depths of soil. The test in this case is designed to determine whether the observed variances can be considered as having been drawn from the same population. 3 of the depths showed homogeneity but the fourth depth (24–36 inches) indicated lack of homogeneity. All of the data were then analyzed by separate depths. The writers are indebted to Dr. F. R. Immer of the Minnesota Agricultural Experiment Station for the method of testing the data and to Dr. A. E. Brandt of the statistical laboratory, Iowa State College, for developing the method and permitting its use.

The nitrate nitrogen content of the first and second 6 inches is important since the roots of the young seedlings evidently have not penetrated below this depth at this early stage of growth. Weaver (*8, pp. 133–134*) described a month-old Marquis wheat plant as follows:

> On May 1, a month after planting and when the second leaf was half grown, a typical root system was drawn. The number of roots varied from three to eight. Lateral roots were fairly abundant but entirely unbranched. The greatest lateral spread was 5 inches and the working depth or working level (*i. e.* a depth to which many roots penetrate and to which depth considerable absorption must take place) 6.5 inches.

The nitrate nitrogen content of the soil in the calcium nitrate plot was significantly higher than that of any of the other plots. The two sets of plots receiving nitrogen as ammonia were significantly higher than the no-treatment plot, but were not significantly higher than the plots receiving manganese sulphate. In the second 6 inches the amount of nitrate nitrogen in the calcium nitrate plots, while still significantly higher than that of the other plots, was lower than that in the first 6 inches. While the ammonium sulphate plot was some-

what higher than the no-treatment plot, it did not differ significantly from the other treatments. The nitrate nitrogen content of the soil in the second foot was, in all cases, higher than that of the second 6 inches. The calcium nitrate plot was significantly higher than the other plots. In the third foot a noticeable increase of nitrogen is apparent. Tests made in 1934 before and after irrigation indicated that the nitrates were washed below the third foot. The first 6 inches; second 6 inches, and second foot were almost depleted as far as the nitrate tests showed. A slight loss was indicated in the third foot.

No significant differences were noted in the first 6 inches or in the second 6 inches of soil taken from the different plots in August (table 5). In the second foot, however, the plot receiving ammonium sulplate differed significantly from the other plots in parts per million of nitrogen. In the 0–36-inch column a significant difference between the no-treatment and the calcium nitrate plots was found. None of the other plots showed a significant difference.

The yield data indicate that the nitrate added as calcium nitrate increased the yield over the other treatments. The soil-analysis data show that the nitrate nitrogen in the calcium nitrate plots was considerably higher in the first 6 inches. Evidently, a lack of nitrogen at this stage affects the normal development of the young seedlings. The calcium nitrate plots were a much darker green than the other plots.

The plots receiving nitrogen as ammonium acid phosphate outyielded the plots receiving no nitrogen. The nitrate content of the first 6 inches of soil, however, was not significantly different from that of some of the plots receiving no nitrogen. A similar condition was found in the second 6 inches.

The ammonium sulphate-treated plots which differed significantly in yield from the plots receiving no nitrogen showed the same condition in the nitrate content of the soil. Since the nitrate was more effective in correcting the chlorotic condition, it would appeor that nitrogen is more readily absorbed by wheat plants as the NO_3 ion than as the NH_4 ion. The benefits received from the ammonium salts could have been due either to nitrification of these salts or to the direct absorption of ammonia. Nitrification could have been appreciable and still not have been detected in the analyses.

Nitrogen Content of Grain and Straw

The nitrogen content of the grain and straw was determined by the Gunning method (*1*).

The average percentage of nitrogen in the grain is given in table 6.

The nitrogen content of the threshed grain varied slightly for the different treatments. The calcium nitrate plots gave the lowest content in every case, except in 1935. The average content for the 3-year period indicates a significant difference over the no-treatment plots for the plots receiving manganese sulphate and ammonium phosphate.

The nitrogen in the straw was considerably lower than in the grain (table 6).

The nitrogen in the straw was low in the ammonium-treated plots. No significant difference was noticed between the other treatments. All of the other plots were significantly higher than the ammonium acid phosphate and ammonium sulphate plots.

TABLE 6.—*Average yearly nitrogen content of grain and of straw from the differently treated plots for 1933 to 1935, inclusive*

GRAIN

Treatment	1933	1934	1935	3-year average
	Percent	*Percent*	*Percent*	*Percent*
$MnSO_4$	2.80	2.74	2.58	2.71
$(NH_4)H_2PO_4$	2.79	2.76	2.58	2.71
$FeSO_4$	2.79	2.74	2.58	2.70
$(NH_4)_2SO_4$	2.79	2.73	2.54	2.68
$CaH_4(PO_4)_2$	2.76	2.73	2.54	2.68
No treatment	2.76	2.73	2.52	2.67
$Ca(NO_3)_2$	2.63	2.73	2.61	2.66
Level of significance	.071	.056	.066	.037

STRAW

No treatment	0.49	0.43	0.52	0.48
$Ca(NO_3)_2$	.45	.46	.56	.48
$CaH_4(PO_4)_2$	.48	.41	.56	.48
$MnSO_4$	.48	.41	.51	.47
$FeSO_4$	.47	.40	.51	.47
$(NH_4)H_2PO_4$	.47	.42	.47	.45
$(NH_4)_2SO_4$	.45	.43	.46	.44
Level of significance	.028	.035	.035	.019

EFFECT OF TEMPERATURE OF IRRIGATION WATER AND FERTILIZER TREATMENTS ON THE DEVELOPMENT OF CHLOROSIS

As previously stated, the mild chlorotic condition often found in small grains after heavy rains or applications of irrigation water to the plants in the earlier stages of growth has been attributed to various causes, one of the commonest being the temperature of the irrigation water. From the tests described above, there is no indication that water at a temperature as low as 40° F. has any more detrimental effect on the plants than water at 60°. The temperature of the soil was affected more by the temperature of the surrounding air than by the temperature of the irrigation water between the range of 40° to 60°.

An application of calcium nitrate immediately after irrigation prevented the occurrence of chlorosis. A similar but less pronounced effect was obtained when ammonium sulphate or ammonium acid phosphate was applied. No difference in the color or type of growth was noticed in the plants receiving manganese sulphate, iron sulphate, and superphosphate. These results indicate that the chlorotic condition resulted from a shortage of nitrogen rather than from any other elemental deficiency. Nitrate proved more effective than ammonium salts in controlling the chlorotic condition, indicating a greater availability of the nitrate ion.

When the nitrate nitrogen content of the soil in the first 6 inches was low, chlorosis resulted. When the content was high, as in the plot treated with calcium nitrate, no chlorosis occurred. Ammonium nitrogen had a similar effect, but to a less degree.

SUMMARY

Tests conducted with Marquis wheat irrigated at germination with 6 inches of water have shown that:

(1) The temperature of irrigation water ranging from 40° to 60° F. has no effect on the yield of grain or straw.

(2) The addition of nitrogen fertilizer to the crop immediately after irrigation prevents the chlorotic condition often observed in young wheat plants after heavy rains or the application of irrigation water and increases the yield of grain.

(3) The irrigation water washes the soluble nitrate nitrogen below the 3-foot level.

(4) Yields both of straw and grain are increased by applications of calcium nitrate, ammonium acid phosphate, and ammonium sulphate in the order named.

(5) In plots treated with calcium nitrate, the nitrate nitrogen content was high in the first 6 inches of soil. In plots receiving no nitrogen the nitrate nitrogen content was significantly lower.

(6) Applications of manganese sulphate, iron sulphate, and superphosphate did not alter the chlorotic condition or increase the yield over the no-treatment plots.

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FACTORS INFLUENCING THE RIPENING SEASON OF SOUR CHERRIES [1]

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INTRODUCTION

With cherries, as with most other fruits, the season at which the fruit matures is important in determining its market value. Thus, varieties such as Black Tartarian, which would be of little worth if their fruit were to ripen 2 weeks later than it does, are highly prized because they are among the first to reach the market.

In a general way each variety has its characteristic ripening season, and efforts to advance, retard, shorten, or lengthen the harvesting season have been limited largely to the selection of varieties that would appear to meet the requirements in question. Nevertheless, there is considerable variation in ripening season within the same variety, not only from place to place but even within the same orchard. Thus in the Graham station orchard near Grand Rapids, Mich., which furnished many of the data for this report, the first picking of Montmorency in 1935 was made on July 16 and the last on September 14. Sometimes this prolonged ripening season occasions no great inconvenience or loss, but at other times it may entail considerable added expense because of the necessity of fractional picking or, if such picking is not employed, it may cause considerable loss in revenue because of the change in price as the season advances, or the lower price received for a product not uniform in degree of maturity.

For commercial canning only cherries that are fully tree-ripened are wanted. Those that are slightly immature lose too much of their color in processing and the appearance of the finished product is impaired. If the fruit is left on the trees until all of it is ripe enough for canning, there is loss from overmaturity and decay, attack by birds, bruising from the wind, wilting, and other causes. There is perhaps a wider range in the degree of maturity that is acceptable for the fresh-fruit trade, but uniformity within the package is highly desirable. Experience shows that in many seasons in certain Montmorency orchards fully 3 weeks intervenes between the first and the last pickings when an effort is made to meet the demands of an exacting trade.

It has seemed desirable, therefore, to make a study of some of the more important factors that influence the season of ripening of cherries and to discover, if possible, practicable means of advancing or retarding and of making more uniform the season of maturity.

REVIEW OF LITERATURE

Though there is a fairly extensive literature pertaining to cherries and cherry culture, data relating to factors other than variety that influence the ripening season are rather limited.

[1] Received for publication Feb. 4, 1937; issued October 1937. Journal article no. 289 (n. s.) from the Michigan Agricultural Experiment Station.

Growers generally are aware of the influence of latitude on the ripening season of fruits. Thus in Michigan the harvesting of Montmorency usually takes place during the first half of July in the more southern counties, during the second and third weeks in the central counties, during the last half of the month in the Traverse City area, and during early August in the northernmost sections that are subject to the influence of Lake Michigan. This difference is considerably greater than the average of 3.6 days retardation in ripening season for each degree of latitude found by Phillips (*9*) [2] as characterizing the Montmorency cherry in the Mississippi Valley. That this influence of latitude on ripening season is probably in large part a temperature effect is indicated not only by the prevailing temperatures at these different latitudes, but by the experience of English and other northwestern European growers who have been able to advance the ripening season of some cherry varieties 2 or 3 weeks by training them as espaliers or cordons along the south side of walls (*11*) and to retard ripening for a similar period by shading (*2*).

With many fruits soil exerts a very pronounced influence on ripening season, light soils tending to promote maturity and heavier soils tending to retard it. This influence is not so marked in the case of cherries, though it has been noted many times (*13*). The application of nitrogenous fertilizers tends to delay maturity in many fruits. This influence has been observed with sour cherries (*12*), but there is some question as to its relative importance. Fertilization has also been noted (*7*) as contributing to the evenness of ripening of cherries. Though rootstocks undoubtedly exert some influence on earliness and evenness of ripening, (*5*), data are not available to indicate clearly what stocks may be used with confidence to advance or to retard ripening.

Little attention has been given to the importance of intravariety strains in the case of cherries, though there are a number of references in the literature to the occurrence of limb sports that differ from the parent tree in ripening season. May Duke in particular has long been noted as a variety characterized by very uneven ripening (*1*, *3*), apparently due to what might be called an ever-sporting tendency, and Hochgenuss von Erfurt (*10*) is apparently another variety of the same type. A number of whole tree and limb variations in season of ripening in the Montmorency variety have been described recently by Drain (*4*), and the presumption is that directly or indirectly other variations of this same type have been responsible for more or less unevenness in ripening in the general run of stock propagated commercially by the nurseries.

MATERIALS AND METHODS

There were available for the purposes of this study the 1931–34 harvesting records of each of 194 cherry trees (*Prunus cerasus* L.) of the Montmorency variety in the so-called Corporation orchard near South Haven, Mich., and the 1931–36 harvesting records of each of 149 trees in the Graham station orchard near Grand Rapids, both of which orchards had been set in the spring of 1920. The records for the Corporation orchard included individual tree yields each year and a series of notes made during the ripening season in which each tree

[2] Reference is made by number (italic) to Literature Cited, p. 532.

was classified as ripening its fruit evenly or unevenly and relatively early, midseason, or late in the season. In the Graham station orchard fractional picking was employed each year on those trees that ripened unevenly and records were kept of the quantities harvested at each picking. Special effort was made each year to harvest the fruit at as nearly the ideal stage of maturity as possible. Measurements were made of the trunk circumference of each tree in the two orchards each autumn, and the crops of individual trees were classified as small, medium, large, or very large if they totaled as follows for trees of the following trunk circumferences (as measured in the fall of 1933):

Trunk circumference (inches):	*Yield (pounds) and crop classification*
Less than 19	Less than 25, small.
Do	25–75, medium.
Do	More than 75, large.
Do	More than 150, very large.
19–23	Less than 75, small.
19–23	75–100, medium.
19–23	125–200, large.
19–23	More than 200, very large.

This is, of course, an arbitrary classification, but it permits a grouping that brings out the influence of relative size of crop on season of maturity.

In addition to the 149 trees at the Graham station already referred to there were a number of others in other blocks, some younger and some older, which were submitted to various experimental treatments and on which records relating to season of maturity were obtained.

One series of records was obtained in 1933 from four representative individuals in a block of fully mature Early Richmond trees in the orchard of the W. R. Roach Co., of Hart, Mich. These trees were large for their age, healthy, and at an earlier stage in their development had been very vigorous. For several years before the records were taken they had been bearing very heavy crops, so heavy in fact that a considerable percentage of the crop had not been sizing up and ripening properly. Fractional picking had been employed each year, but part of the crop never reached a degree of maturity that made it acceptable for commercial canning, even though allowed to remain on the trees until after the Montmorency harvest had been completed.

Temperature data to compare with the fruiting records of the Graham station trees were obtained from the Grand Rapids office of the United States Weather Bureau, located about 4 miles from the orchard.

INFLUENCE OF TEMPERATURE AND SIZE OF CROP ON RIPENING SEASON

Perhaps the most obvious variation in ripening period encountered in this study was that associated with season. In 1931, 1935, and 1936 cherries ripened late, in 1932 relatively early, and in the other years about midseason. The ripening season in 1934 was very uniform; in 1935 and 1936, uneven and long drawn out, and in the other years intermediate. These facts are well brought out in figure 1 and table 1 for the trees in the Graham station block.

The seasonal variations in earliness or lateness of ripening can be explained in part by the accompanying temperatures. The three relatively early seasons, 1932–34, were characterized by at least moderately high mean May, June, and July temperatures and by corre-

spondingly high heat accumulations (measured by daily maxima) during these months. Though the mean July temperatures and July heat accumulations of the 3 late-maturing years were as high as or higher than those of the 3 early-maturing years, May temperatures and heat accumulations were markedly lower in 1931 and 1935 and June temperatures were markedly lower in 1935 and 1936. The fruit, therefore, had advanced considerably further in its development by July 1 in some years than in others.

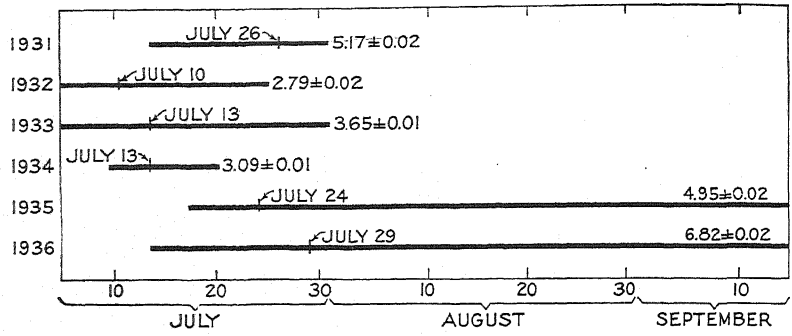


FIGURE 1.—Ripening season of fruit on 149 trees at the Graham station, Grand Rapids, Mich., 1931–36. Vertical lines indicate mean picking dates; figures at right end of horizontal lines indicate standard deviations, in days, in harvesting dates.

TABLE 1.—*Yields and ripening dates of 149 Montmorency cherry trees at the Graham station, 1931–36, and temperature records for the growing seasons of those years*

Year	Average yield per tree	First picking	Last picking	Average harvest date	Standard deviation in ripening date	Mean temperature			Heat accumulation [1]		
						May	June	July	May	June	July
	Pounds				*Days*	°F.	°F.	°F.	°F.	°F.	°F.
1931	130.6	July 14	July 31	July 26	5.17±0.02	56.0	71.3	75.8	744	1,188	1,379
1932	44.7	July 5	July 23	July 10	2.79±0.02	59.4	70.5	73.0	855	1,170	1,280
1933	109.4	---do-----	Aug. 1	July 13	3.65±0.01	59.7	74.0	75.6	833	1,290	1,373
1934	155.8	July 9	July 19	---do----	3.09±0.01	63.7	73.3	76.8	1,050	1,287	1,450
1935	100.2	July 17	Sept. 14	July 24	4.95±0.02	53.3	64.8	76.5	644	948	1,360
1936	242.7	July 14	---do-----	July 29	6.82±0.02	62.9	66.2	77.3	1,001	1,101	1,472

[1] Heat accumulation figured as the total of the day degrees of maximum temperature exceeding 41° F.

Examination of the data presented in table 1, however, suggests that size of crop may have had some influence on season of maturity. Thus in 1936, when very heavy crops were borne, the maturing season was very late; and in 1931, another year of fairly heavy crops, the ripening season was again late. The years 1932 and 1933 were years of light or moderate production and the ripening seasons were comparatively early. On the other hand, in 1934 the trees bore heavier crops than were borne the two preceding seasons and they matured their fruit as late or slightly later even though temperatures were higher, while in 1935 with only a medium crop they matured their fruit late. The influence of size of crop upon season of ripening is more clearly indicated by the data in table 2, in which the harvesting records for several seasons of the individual trees in the Corporation and Graham station orchards are classified as to size, earliness, and evenness of ripening.

It will be noted that in both orchards there was a marked tendency for trees bearing relatively light crops to mature their fruit early and for the fruit to ripen evenly, whereas the trees bearing relatively heavy crops matured their fruit late and the fruit ripened more or less unevenly. Trees with medium-sized crops were intermediate in respect to both time and uniformity of ripening. This influence of size of crop is given a quantitative expression by the data presented in table 3. Presumably the retarding effect of a heavy crop was more important in causing late ripening in the Graham station orchard in 1936 than the somewhat subnormal mean temperature during June, for temperatures during the remainder of the growing season were up to or above the average.

TABLE 2.—*Classification of the individual tree yields on a basis of ripening season, crop yield, and manner of ripening, in Corporation and Graham station orchards*

[Each tree each year was classified as bearing a light, medium, heavy, or very heavy crop; as having an early, midseason, or late ripening season; and as having fruit that ripened evenly or unevenly]

CORPORATION ORCHARD, 1931–34 [1]

Kind of crop and manner of ripening	Trees	Percent of total	Kind of crop and time of ripening	Trees	Percent of total
	Number			*Number*	
Light:			Light:		
Even	304	83	Early	115	43
Fairly even	12	3	Midseason	145	55
Uneven	51	14	Late	6	2
Total	367	100	Total	266	100
Medium:			Medium:		
Even	129	45	Early	35	14
Fairly even	84	29	Midseason	188	76
Uneven	73	26	Late	25	10
Total	286	100	Total	248	100
Heavy:			Heavy:		
Even	8	9	Early	0	0
Fairly even	22	26	Midseason	27	56
Uneven	55	65	Late	21	44
Total	86	100	Total	48	100

GRAHAM STATION ORCHARD, 1931–36

Light:			Light:		
Even	129	89	Early	89	61
Fairly even	10	7	Midseason	52	36
Uneven	6	4	Late	4	3
Total	145	100	Total	145	100
Medium:			Medium:		
Even	156	62	Early	115	46
Fairly even	43	17	Midseason	122	48
Uneven	52	21	Late	14	6
Total	251	100	Total	251	100
Large:			Large:		
Even	171	52	Early	55	17
Fairly even	62	19	Midseason	172	53
Uneven	94	29	Late	100	30
Total	327	100	Total	327	100
Very large:			Very large:		
Even	8	5	Early	2	1
Fairly even	61	38	Midseason	83	52
Uneven	92	57	Late	76	47
Total	161	100	Total	161	100

[1] In 1931 the crops in the Corporation orchard were not classified as to early, midseason, or late and again in 1934 only a part was so classified.

TABLE 3.—*Ripening-season data for individuals of selected groups of Montmorency cherry trees in the Graham station orchard, 1931–36*

[The figures presented are the averages for the 25 lowest and 25 highest yielding individuals of each year, regardless of what those individual trees may have yielded in other years. The 50 individuals were the extremes from the standpoint of yield of the 149 trees in the orchard]

Year	The 25 lowest-yielding trees [1]			The 25 highest-yielding trees [1]		
	Average yield	Average ripening date	Averages of standard deviations in ripening date for each of the 25 trees	Average yield	Average ripening date	Averages of standard deviations in ripening date for each of the 25 trees
	Pounds		*Days*	*Pounds*		*Days*
1931	59	July 19	4.80	195	July 28	4.01
1932	7	July 8	2.44	97	July 10	3.76
1933	49	July 11	1.70	175	July 14	2.10
1934	46	July 10	----------	262	July 16	.40
1935	48	July 23	1.25	165	July 25	1.30
1936	125	July 27	4.20	346	July 29	5.30

[1] It should be made clear that each of the 25 trees (say lowest-yielding trees) had a day which was considered to be its "ripening" date (here an average) and that each tree had a "ripening period" standard deviation, or a period in which about two-thirds of its fruit ripened. The averages are merely the averages of these quantities for the 25 trees. Hence the average of the standard deviations is an average of the periods, for each tree, in which about two-thirds of the fruit ripened. This value shows the average period in which about two-thirds of the cherries ripened; it is akin to an average of ripening periods or an average of ranges. This average of the standard deviations is not a standard deviation of the set of items made up of averages of ripening dates.

INFLUENCE OF SHADING AND SIZE OF CROP ON RIPENING SEASON

It has long been a matter of observation that cherries in the tops and on the more exposed outside limbs of the trees often ripen earlier and more evenly than those on the lower and interior limbs. In order to obtain information as to whether this is in the main a size-of-crop or a shading-and-exposure influence several trees were selected shortly before harvest in the summer of 1932 and again in 1933, as follows: (1) Trees with a heavy crop on one side and a light crop on the other, (2) trees with a heavy crop in the top part and a light crop in the bottom, but with no marked difference between the two parts in exposure to light. Fractional pickings were made of the two sides of these trees and of their upper and lower halves, respectively, and great care was taken to harvest fruit only of the same degree of maturity. Still other trees were selected in the same years, all of which were bearing relatively heavy crops, but some of which had (3) dense tops with heavily shaded centers and (4) others which had very open spreading tops with centers well exposed to sunlight. In harvesting the fruit from these last two groups, fractional pickings were made of (*a*) tops and centers and (*b*) inside or center portions. The harvesting records of these four groups of trees are presented in table 4.

TABLE 4.—*The ripening season and yield of Montmorency cherry trees as influenced by the location of the fruit in different parts of the tree, Graham station orchard, 1932–33*

Tree no.	Picking dates	Portion of tree	Yield		Tree no.	Picking dates	Portion of tree	Yield	
			Lb.	*Pct.*				*Lb.*	*Pct.*
15–A	July 8, 1932	East side	39	76	1–C (open top)	July 9, 1932	Outside	36	84
	July 11, 1932	do	12	24		July 12, 1932	do	7	16
	July 8, 1932	West side	14	88		July 8, 1932	Inside	9	50
	July 11, 1932	do	2	12		July 12, 1932	do	8	44
	July 6, 1933	East side	8	11		July 15, 1932	do	1	6
	July 11, 1933	do	48	65		July 7, 1933	Outside	21	19
	July 17, 1933	do	18	24		July 12, 1933	do	51	47
	July 6, 1933	West side	35	69		July 16, 1933	do	37	34
	July 11, 1933	do	16	31		July 12, 1933	Inside	12	50
1–H	July 8, 1932	Top	41	77		July 16, 1933	do	12	50
	July 11, 1932	do	12	23	1–D (dense top)	July 7, 1933	Outside	16	26
	July 8, 1932	Bottom	7	37		July 12, 1933	do	38	61
	July 11, 1932	do	12	63		July 16, 1933	do	8	13
1–B (dense top)	July 9, 1932	Outside	60	75		July 12, 1933	Inside	22	45
	July 12, 1932	do	20	25		July 16, 1933	do	27	55
	July 9, 1932	Inside	2	8	8–H (open top)	July 7, 1933	Outside	17	17
	July 12, 1932	do	20	77		July 12, 1933	do	72	71
	July 15, 1932	do	4	15		July 16, 1933	do	12	12
	July 7, 1933	Outside	9	8		July 12, 1933	Inside	30	59
	July 12, 1933	do	72	69		July 16, 1933	do	21	41
	July 16, 1933	do	24	23	9–J (open top)	July 7, 1933	Outside	5	5
	July 12, 1933	Inside	35	58		July 12, 1933	do	60	66
	July 16, 1933	do	25	42		July 16, 1933	do	26	29
						July 12, 1933	Inside	18	60
						July 16, 1933	do	12	40

It will be noted that in both years the light-cropping side of tree 15–A matured its fruit somewhat earlier and more evenly than the heavier cropping side. However, both trees (1–H and 15–A) which had been so grown and so pruned that both tops and bottoms were well exposed to light but which bore heavier crops in the tops than in the bottoms, had a tendency to mature their fruit earlier in the tops than in the bottoms in spite of the larger crops. This might lead to the assumption that shading of fruit and foliage in the lower part of the tree was responsible for the later and more uneven ripening. However, the fact that those trees for which "outside" and "inside" picking records were obtained ripened their outside fruit earlier and more evenly than their inside fruit, regardless of whether the tree had a dense or an open top, would indicate that shading within the limits commonly afforded by the tops of cherry trees is perhaps not of so great importance in this connection. Observations as to the distribution and ripening of the fruit in the several parts of these trees, together with the data in table 6, lead unmistakably to the opinion that both earliness and evenness of ripening depended more upon the relative amount of leaf area as compared with the amount of fruit borne by the portions of the tree in question. Where there was a relatively large amount of foliage for the fruit of the limb or part of the tree in question, the ripening season was comparatively early and even; where foliage was limited for the fruit associated with it and to the development and ripening of which it was contributing, ripening was delayed and uneven. This interpretation of ripening behavior ties in with and helps explain some of the seasonal peculiarities in season of maturity mentioned earlier in this paper, e. g., the late and uneven ripening of the 1936 crop, which was extremely heavy.

Even more convincing as to the retarding effect on ripening of a very large crop and of limited leaf surface are the records obtained in 1933 in the Early Richmond block briefly described under Materials and Methods. Four trees considered as fairly representing the conditions of tree growth and crop in this block were selected. At harvest counts were made of the number of cherries on limbs of approximately the same diameter in each of these trees and of the number of leaves accompanying them. The cherries were weighed and classified as to maturity, and leaf areas were determined. Similar records were made for the whole trees. The data obtained are summarized in tables 5 and 6. In general the larger the amount of foliage in relation to the amount of fruit, the more even and complete was the ripening of the fruit. It is realized, of course, that branches of the sizes included in this table are not entirely independent, for there is considerable translocation of food materials from limb to limb in the cherry tree (*8*). The data obtained permit no exact statement as to the number of leaves or the leaf area necessary to mature a cherry, but for Early Richmond in the Roach orchard in 1933 it appears that not less than 5 square inches of leaf area was required for each fruit if it was to ripen properly and that when there was an 8- or 10-square-inch leaf area present per cherry, the chances of those cherries ripening properly at the normal maturing season were good.

TABLE 5.—*Harvesting and leaf records of 4 Early Richmond trees growing near Hart, Mich., arranged in descending order according to apparent vegetative vigor, 1933*

Item	Tree A	Tree B	Tree C	Tree D
Trunk circumference.....inches..	24.5	20.0	20.5	24.0
Leaves, estimated [1].....number..	89,000	68,000	63,000	64,000
Leaf area, estimated [2].....square inches..	293,700	223,000	195,900	202,200
Leaves per fruit, estimated.....number..	3.1	3.2	1.10	1.15
Leaf area, per fruit, estimated.....square inches..	10.30	10.60	6.63	3.66
Total fruits.....number..	28,285	20,976	29,559	55,350
Fruits maturing properly.....do....	18,586	11,149	13,753	16,850
Fruits not maturing properly.....do....	9,699	9,827	15,806	38,500
Total weights of fruit.....pounds..	178.3	138.4	157.6	266.0
Weight of fruit maturing properly.....do....	117.9	72.4	81.1	91.5
Weight of fruit not maturing properly.....do....	60.4	66.0	76.5	174.5
Average cherries per pound.....number..	159	152	188	203
Average cherries per pound maturing properly.....do....	158	154	170	184
Average cherries per pound not maturing properly.....do....	160	150	207	221

[1] Estimate made by counting the leaves on several branches 2 inches in diameter and multiplying that number by the estimated number of branches of equivalent size.

[2] Estimate made by multiplying the estimated number of leaves by the average area of a large random sample.

TABLE 6.—*Size and maturity of fruits from selected branches of Early Richmond trees, as influenced by the associated leaf area, Hart, Mich., 1933*

Tree	Branch no.	Branch diameter	Fruits maturing properly		Average number of fruits per pound maturing properly	Fruits not maturing properly		Average number of fruits per pound not maturing properly	Total leaves	Average number of leaves per fruit	Total leaf area	Average leaf area per fruit	Fruits maturing properly
		In.	*Number*	*Lb.*	*Number*	*Number*	*Lb.*	*Number*	*Number*	*Number*	*Sq. in.*	*Sq. in.*	*Percent*
A	1	¾	562	3.3	170	31	0.2	155	1,265	2.1	3,767	6.4	95
	5	¾	320	1.95	164	5			700	2.1	2,205	6.8	99
	6	¾	515	3.15	164	69	.4	172	1,808	3.0	5,243	9.0	88
	7	¾	348	2.0	174	123	.75	164	1,126	2.3	3,772	8.0	73
	2	¼	91	.55	165	14	.07	200	209	2.0	587	5.6	86
	3	¼	83	.5	166	10	.05	200	336	3.6	1,014	10.9	89
	4	¼	103	.65	159	0	0		182	1.76	551	5.3	100
	8	¼	48	.3	160	21	.1	210	148	2.1	534	7.7	70
B	1	¼	84	.5	168	30	.15	200	222	1.9	672	5.9	74
	5	¼	53	.3	177	26	.15	173	234	2.96	720	9.1	67
	7	¼	75	.45	167	45	.25	180	157	1.3	455	3.8	63
	3	⅛	83	.5	166	19	.1	190	219	2.1	650	6.3	81
	2	¾	226	1.4	161	195	1.1	177	454	1.07	1,384	3.3	54
	4	¾	231	1.5	154	30	.2	150	701	2.7	2,046	7.8	89
	6	¾	177	1.2	148	56	.4	140	495	2.1	1,494	6.4	76
C	1	¼	70	.4	175	56	.15	373	141	1.1	400	3.2	55
	2	¼	73	.4	182	98	.5	196	214	1.2	588	3.4	43
	4	¼	37	.2	185	71	.25	284	106	.98	289	2.5	34
	5	¼	78	.4	195	76	.3	253	202	1.3	559	3.6	51
	3	¾	185	1.1	168	189	.95	199	490	1.3	1,465	3.9	50
	6	¾	334	1.9	176	424	1.9	223	1,012	1.3	2,742	3.6	44
	7	¾	255	1.55	165	217	1.2	181	735	1.5	2,249	4.8	54
	8	¾	407	2.45	166	336	2.0	168	1,418	1.9	4,296	5.8	55
D	2	¼	106	.5	212	39	.2	195	259	1.78	880	6.1	73
	3	¼	26	.2	130	114	.6	190	277	1.9	781	5.6	18
	4	¼	39	.15	260	143	.7	204	204	1.1	669	3.7	21
	7	¼	108	.55	196	54	.2	270	153	.94	430	2.7	66
	1	¾	201	1.0	201	676	3.1	218	712	.8	2,065	2.4	22
	5	¾	562	2.8	200	314	1.5	210	816	.93	2,407	2.7	64
	6	¾	238	1.2	198	492	2.15	229	509	.7	1,598	2.2	32
	8	¾	397	1.95	204	256	1.1	233	747	1.1	2,181	3.3	60

[1] Correlation coefficient between average leaf area in square inches per fruit and percentage of fruit maturing properly equals 0.64±0.04.

EFFECT OF NITROGENOUS FERTILIZERS ON RIPENING SEASON

The statement is often made that applications of nitrogen-carrying fertilizers have a tendency to delay the ripening of cherries, and many fruit growers are of that opinion. All of the trees furnishing records for this study received annually a moderate application of sulphate of ammonia—from 2 to 4 pounds per tree—the quantity varying with the season and size of tree. The records of these trees therefore did not furnish an answer to the question as to the influence of nitrogen-carrying fertilizers on season of maturity of the fruit. Inasmuch as an earlier study (*6*) of cultural practices in the Montmorency cherry orchard, which included some fertilizer trials, had failed to show any marked influence of applications of nitrogenous fertilizers on ripening season, it was decided to put the idea to a crucial test. Several trees on the Graham station grounds, not included in the block for which ripening-season records have been given, several years older but more or less comparable to them, were treated with heavy applications of sulphate of ammonia each year for 3 successive years. Eighteen pounds per tree per year were applied to some and 25 pounds per tree

to others. These applications were so heavy that they destroyed all vegetation beneath the trees, but apparently they did not injure the tree roots, at least not seriously, for the trees made a very vigorous vegetative growth, blossomed freely, and set and matured heavy crops. Each year the fruit ripened midseason or earlier for Montmorency in that part of the State and the ripening season was remarkably even. The heavy fertilizer applications had no retarding effect on maturity. There was ample foliage throughout the trees for the even and reasonably early maturity of all the fruit that they produced.

In this connection it should be mentioned that the thin, weak wood had been removed from the centers of these trees so that they contained few or no fruiting branches that had set a considerable number of fruits but that did not possess a correspondingly large amount of foliage. Observation leads to the opinion that the idea that the application of nitrogenous fertilizer to cherry trees causes delayed maturity and uneven ripening is due in large measure to the fact that because of the crowding and shading effect of the vigorous branches in the outside and top of the tree, the interior branches become weak, produce little foliage, and then set more fruit than they can mature properly. The result is delayed and uneven ripening on the part of this fruit in the center of the tree. Removal of the slender, weak fruiting wood from the centers would do away with the difficulty.

INFLUENCE OF STRAIN ON EARLINESS AND EVENNESS OF RIPENING

Reference has been made to the occurrence of late-maturing limb or whole-tree sports in cherry orchards. The Corporation orchard, which furnished part of the data for this study, contained one such late-ripening tree. In no year could it be picked when the other trees were being harvested, its harvesting season being about 10 days later. This particular tree has been propagated and some of its progeny brought to bearing age. The daughter trees faithfully reproduce the late-maturing characteristics of their parent, thus furnishing evidence that the late ripening of the parent tree is not due to stock or other environmental influences, but is due to a strain difference. The Graham station orchard likewise contains one late-maturing whole-tree sport, averaging about a week later than the other trees in the same block, and several late-maturing limb sports. The author has noted many such late-maturing whole-tree and limb sports in the cherry orchards of Michigan. They are of frequent occurrence in Montmorency, very frequent occurrence in English Morello, of less frequent occurrence in Early Richmond. A large number of these season-of-maturity sports have been propagated at the Graham station, and they have been found to transmit their characteristics to their vegetative progeny. There can be no question, therefore, as to the existence of late-maturing and early-maturing strains of cherries in the stocks of the three leading sour cherry varieties as they are obtainable from the trade, and there can be no question as to how they have originated. Close observation in hundreds of cherry orchards in Michigan warrants the statement that, though these striking sports are of rather frequent occurrence, they are recognized by the grower for what they are, they are not harvested along with the main crop, in many cases they are not harvested at all, and perhaps on the whole they can be ignored.

The occurrence of these striking season-of-maturity sports, however, raises a question as to whether there are not an equal or greater number of less prominent strains that are not so readily recognized but that, commercially, are more important because they are constantly contributing to lateness or unevenness of maturity. Obviously the very limited number of trees for which harvesting-season records were available for this study are inadequate for a satisfactory answer to this question. It may be noted, however, that, except for the two late-maturing whole-tree sports just mentioned, in no single instance did a tree that ripened its fruit relatively early or relatively late in some one season similarly ripen its fruit relatively early or relatively late in all of the other seasons. Thus, for instance, of the 17 trees out of the entire 149 in the Graham station orchard that matured their fruit earliest in 1931, only 4 matured their crop relatively early in 1932, only 2 in 1933, 7 in 1934, 2 in 1935, and 2 in 1936; furthermore, 11 of the 17 matured their fruit relatively late in 1932, 1 matured its fruit late in 1933, 2 in 1934, 4 in 1935, and 2 in 1936. The influence of the ratio between amount of foliage and size of crop, already discussed, apparently was sufficient to mask any minor strain influences in respect to ripening season.

DISCUSSION

The interpretation to be placed on the data that have been presented is that, though the ripening season of cherries depends in large measure upon growing-season temperatures, both earliness and evenness of ripening are considerably influenced by size of crop and the relative amount of leaf surface that accompanies it. Ripening can be hastened and rendered more uniform by employing such cultural measures as promote a vigorous growth and keep the leaves functioning actively. Pruning, especially the kind that removes weak, slender, interior limbs that are rather poorly supplied with leaves, and the liberal use of nitrogenous fertilizer tend to provide such proper balance between vegetative growth and size of crop, and spraying with such materials as protect the leaves from fungous attack without causing an undue amount of spray injury tends to maintain it.

SUMMARY

The ripening season of cherries in contiguous areas often varies greatly, there being as much as 3 to 4 weeks' difference for the same variety between places 25 to 200 miles apart in the western Michigan fruit belt.

The ripening season of the same variety in the same orchard may vary as much as 2 to 3 weeks from year to year.

Growing-season temperature is a factor of major importance in determining the ripening season of cherries, ripening being delayed by relatively low temperatures.

Variation in ripening season within the same orchard in any one season and likewise from year to year is due in large measure to size of crop and the relative amount of foliage associated with that crop, large crops and limited foliage tending to delay maturity and cause uneven ripening, and, conversely, small crops and abundant foliage tending to promote earliness and cause even ripening.

Cultural measures that tend to establish and maintain a proper balance between crop and vegetative growth tend toward earlier and more even maturing. Such measures include the use of nitrogenous fertilizers, spraying to protect the leaves, and the removal by pruning of small, interior limbs that have a relatively small amount of leaf surface in comparison to their crop.

Contrary to general opinion, the liberal use of nitrogen-carrying fertilizers was not found to lead to delayed maturity and uneven ripening. It has the opposite effect, if proper pruning methods are employed.

Bud sports giving rise to late- or early-maturing strains are of rather frequent occurrence in the sour cherry, but they are of secondary importance to the factors already mentioned in causing delayed or uneven ripening in the crop as a whole.

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OBSERVATIONS ON THE VARIATIONS IN CYANOGENETIC POWER OF WHITE CLOVER PLANTS [1]

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INTRODUCTION

Mirande [2] appears to have been the first to note the cyanogenetic property of some individuals of the common white clover, *Trifolium repens* L., which is abundant in lawns and pastures. He barely anticipated Armstrong, Armstrong, and Horton [3] in the announcement of the observation. The last-named authors were seeking to explain the notable differences in nutrient qualities of some adjacent pastures. Because the botanical composition of the pastures was not alone sufficient to explain the observed differences they sought qualitative differences in plants as well as possible peculiarities of the soil to explain the discrepancies. They stated that a kind of "wild white clover" was coming into favor early in the century because of its greater longevity and value in pastures, and that many such plants were cyanophoric, whereas cyanophoric seedlings of the common white clover were rare. Their observations showed that white clover plants from 15 sources varied both in cyanogenetic power and in ability to hydrolyze cyanogenetic glucosides when such glucosides were added to aqueous extracts of the clovers. Only 2 of the 15 groups tested were found to act selectively on different cyanophoric glucosides, the remainder acted either on all or on none of the glucosides.

More recently Askew [4], Doak [5], and Rigg et al. [6] have studied the cyanophoric properties of white clovers in New Zealand. Like Armstrong and his associates, they found that the wild white clovers were cyanophoric, but that the clovers' cyanophoric powers varied widely with location and type. Askew found a seasonal variation in the hydrocyanic acid (HCN) content of white clover, with a tendency for the acid to increase in late summer. Doak [5] concluded that:

> There is considerable correlation between HCN content and type of white clover.
>
> The most highly producing and persistent lines are invariably associated with high HCN content while the poorer short-lived types are low in this respect.

Doak stated also that plants from the same line vary in HCN content. Rigg, Askew, and Kidson [6] found a variation of from 0.0016 to 0.0124 percent of HCN in white clover from 11 sources.

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[2] MIRANDE, M. SUR LA PRÉSENCE DE L'ACIDE CYANHYDRIQUE DANS LE TRÈFLE RAMPANT (TRIFOLIUM REPENS L.) Compt. Rend. Acad. Sci. [Paris] 155: 651–653. 1912.

[3] ARMSTRONG, H. E., ARMSTRONG, E. F., and HORTON, E. HERBAGE STUDIES. II.—VARIATION IN LOTUS CORNICULATUS AND TRIFOLIUM REPENS (CYANOPHORIC PLANTS). Roy. Soc. [London], Proc., B 86: 262–269. 1913.

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[5] DOAK, B. W. A CHEMICAL METHOD FOR THE DETERMINATION OF TYPE IN WHITE CLOVER. New Zeal. Jour. Sci. and Technol. 14: 359–365, illus. 1933. See p. 364.

[6] RIGG, T., ASKEW, H. O., and KIDSON, E. B. OCCURRENCE OF CYANOGENETIC GLUCOSIDES IN NELSON PASTURE PLANTS. New Zeal. Jour. Sci. and Technol. 15: 222–227. 1933.

In 1934, when the New Zealand work first came to the writer's attention, tests of 15 plants of white clover for HCN had proved to be all negative, but potted New Zealand white clover seedlings were consistently cyanogenetic. Early in May 1935, preliminary tests of 30 clover plants growing on the campus of University Farm, St. Paul, Minn., showed 6 to be strongly cyanogenetic. Five hundred plants were permanently labeled by June 24, 1935. This study was continued until early in August when further tests for 1935 had to be abandoned.

TECHNIQUE AND RESULTS

The technique employed in the tests was as follows: Six mature leaves (18 leaflets) of each clover plant were put into a 6- by ¾-inch test tube, frozen at −15° C. Nearly saturated picric acid (0.05 molar) was poured over sodium bicarbonate until all of the solid dissolved. If any precipitate formed within 2 minutes more picric acid was added. A layer 2 mm deep was poured into a Petri dish, and white filter paper in strips 3 mm wide was momentarily dipped straight down into the solution, then laid on glass rods for the solution to soak up the paper and partly dry out.

One milliliter of water was measured from a burette into each tube containing frozen clover leaves. The test strip was inserted into the tube, and held in place by the cork. These were allowed to stand at room temperature (22° to 28° C.) for 1 day, when they were set into water at 50° for 15 minutes to volatilize HCN in the solution. The papers were removed and compared immediately with the standards set for scaling purposes. This is a modification of the Guignard [7] test.

As interest was centered in the groups of cyanophoric plants rather than in the exact amount of hydrocyanic acid in each plant, the test papers which indicated the unquestioned presence of hydrocyanic acid in the clover plants were separated into only five groups (table 1). The records entered in table 1 under "Trace" indicate a color of the paper between that of a negative test (paper the original yellow) and definitely reddened tinge to the yellow or strongly colored paper, which is taken as proof of presence, and a measure of quantity of hydrocyanic acid present.

The conditions of soil, moisture, and light exposure, although not identical within each plot, were much alike.

It is seen from table 1 that the greater portion of the plants in any location and at any time were negative in tests for hydrocyanic acid. About two-thirds of the total number tested were negative. It might be expected that these tests were grouped largely about some plants consistently negative in all tests. Plants consistently negative were grouped together, as were also those consistently positive and those intermediate ones which showed both negative and positive reactions. The number of tests made on each plant is given in table 2, and the plant is entered in the line corresponding to number of tests. Thus, if a plant was tested five times and found to be positive three times and negative twice, it would be entered in the column for positive three times. When only a trace appeared one or more times, the plant would be entered in the column headed "Trace." Such

[7] GUIGNARD, L. LE HARICOT À ACIDE CYANHYDRIQUE, PHASEOLUS LUNATUS L. Compt. Rend. Acad. Sci. [Paris] 142: 545-553. 1906.

plants are essentially negative. All groups of plants were tested at least three times, but in each group some plants were lost by the destruction of stakes, and other plants had to be assigned the same number. This accounts for the 39 plants tested less than three times, and 133 plants tested three times.

TABLE 1.—*Distribution of hydrocyanic acid in clover plants according to location of plants*

Plant group nos.[1]	Number of tests		Plants testing—						
	Total	Per plant	Negative	Positive (classified as to approximate intensity) [2]					
				Trace	1	2	3	4	5
0–99	420	5	278	13	45	46	23	13	2
100–199	388	4	289	23	34	18	20	2	2
200–299	299	3	204	18	36	19	14	8	0
300–399	384	4	217	13	48	57	40	8	1
400–499	492	5	297	21	79	47	28	12	8
Total	1,983		1,285	88	242	187	125	43	13
Percentage of total tests			64.80	4.4	12.20	9.43	6.30	2.17	0.66

[1] Site of plants and soil types: 0–99, highland, various types of soil and moisture, old and young plants; 100–299, flat, shallow, sandy soil over gravel bed, old stand; 300–399, sloping sandy soil, thin sod 7 years old; 400–499, black soil, bottom land of pasture, old sod.

[2] "Trace" indicates a color of the paper in between definite negative and definite proof of the presence of hydrocanic acid in appreciable quantity; classifications 1 to 5 indicate hydrocyanic acid in increasing quantities.

TABLE 2.—*Consistency in the cyanogenetic property of white clover plants as measured by the Guignard test-paper method* [1]

Plants tested (number)	Tests	Plants testing—													
		Negative		Positive (classified as to approximate intensity)											
				Trace		1		2		3		4		5	
	Number	*Number*	*Percent*	*Number*	*Percent*	*Number*	*Percent*	*Number*	*Percent*	*Number*	*Percent*	*Number*	*Percent*	*Number*	*Percent*
149	5	53	35.6	2	1.34	25	16.8	17	11.4	27	18.1	9	6.04	16	10.7
192	4	82	42.7	10	5.21	34	17.7	26	13.5	21	10.9	19	10.00	------	------
133	3	57	42.9	9	6.77	36	27.1	16	12.0	15	11.3	------	------	------	------
27	2	20	74.1	------	------	4	14.8	3	11.1	------	------	------	------	------	------
12	1	9	75.0	------	------	3	25.0	------	------	------	------	------	------	------	------

[1] GUIGNARD, L. See footnote 7.

The percentages of plants giving a negative reaction in the groups tested five, four, and three times agree rather well. This suggests that there there are mixed in the sods at University Farm a larger portion of noncyanogenetic white clovers than cyanogenetic. There is also a group of plants similar in general appearance but which gives consistently positive tests for HCN. Some plants varied in cyanogenetic power, but the preponderance of plants was consistently noncyanogenetic. Although not specifically shown in tables 2 or 3, there was no detectable consistency among those plants with one or two positive tests for HCN, for many were positive at first, but negative in later tests, and some were negative at first but positive in one or two later tests. Individually variable plants showed no consistency in their tests.

TABLE 3.—*Tests of cyanogenetic power of white clover on University Farm campus, on various dates during the summer of 1935*

Date	Plant group nos.	Total tests	Plants testing as indicated						
			Negative	Positive, classified as to approximate intensity					
				Trace	1	2	3	4	5
May 9	0- 99	91	75	3	6	6	1		
May 13	0- 99	81	62	2	6	8	1	2	
May 19	400-499	99	71	10	14	4			
May 21	0- 99	79	39	2	14	13	8	3	
May 22	400-499	100	61	1	13	15	8	2	
May 24	100-199	100	84	4	3	3	4		2
Period total		550	392	22	56	49	22	7	2
Percentage		100.00	71.28	4.00	10.18	8.91	4.00	1.27	.36
June 7	0- 99	85	46	5	13	9	7	3	2
Do	400-499	99	64	8	14	8	3	1	1
June 19	100-199	96	73	8	7	4	3	1	
Do	200-299	100	63	9	13	6	4	5	
June 24	300-399	99	77	2	3	9	8		
June 27	100-199	100	77	5	8	5	4	1	
Do	200-299	99	89	0	2	3	4	1	
July 3	300-399	98	39	3	11	23	18	3	1
Do	400-499	98	43	0	23	13	7	5	7
Period total		874	571	40	94	80	58	20	11
Percentages		100.00	65.33	4.58	10.76	9.15	6.63	2.29	1.26
July 16	0- 99	84	56	1	6	10	6	5	
Do	300-399	97	47	2	18	15	11	4	
July 29	200-299	100	52	9	21	10	6	2	
July 30	100-199	92	55	6	16	6	9		
July 31	400-499	96	58	2	15	7	10	4	
Aug. 1	300-399	99	54	6	16	10	3	1	
Period total		559	322	26	92	58	45	16	0
Percentages		100.00	57.60	4.65	16.46	10.38	8.05	2.86	
Grand total		1,983	1,285	88	242	187	125	43	13
Percentage		100.00	64.80	4.44	12.20	9.43	6.30	2.17	.66

Although this work was stopped about the middle of the normal Minnesota growing season, the dry, hot weather after good spring and early summer rains permitted the observation of the effect of unfavorable growing conditions on the cyanogenetic activity of the clovers. Tests were made from May 9 to August 1, 1935, as shown in table 3, where the dates and number of tests of each group are tabulated against the progress of the season.

From the data in table 3 which lists the tests in calendar order one notes a tendency for the number of negative tests of all groups to decrease. Subtotals are taken to show the possible differences in groupings with the advance of the season, utilizing the greatest intervals between groups of tests and changes in weather. The first group of tests was made during cool, moist spring weather; the second or middle group during hot, humid weather when vegetation was flourishing; and the last during a severe drought.

The percentage of negative tests decreased appreciably with the advance of the season from 71.28 to 57.60. There was some rise in the number of plants mildly cyanogenetic, and a tendency to maintain or increase throughout the cyanogenetic activity in all plants but

those highest in HCN. This observation seems to confirm those of Askew.[8]

If the idea held by many, that cyanogenetic activity is induced or accelerated by conditions unfavorable for gross plant growth then the conditions of July should have induced even greater cyanogenetic activity than is apparent, as the increase over the second period is only slightly greater than the increase of the second over the first. The clover plants in many cases were badly stunted by the time the last test material was taken.

SUMMARY AND CONCLUSIONS

To determine the cyanogenetic property of white clover (*Trifolium repens* L.), from 1 to 5 individual tests were made on 513 different white clover plants. In all, 1,983 separate tests were performed between May 9 and August 1, 1935, of which about two-thirds were negative.

Approximately 40 percent of the plants tested three or more times were consistently negative.

Only 10 percent of the plants tested three or more times were consistently positive, and these were usually strongly positive.

There is an indication of increase in the cyanogenetic power of plants with increase in size of plant, with progress of season, and with a generally decreased moisture supply.

[8] ASKEW, H. O. See footnote 4.

MACROSPOROGENESIS AND EMBRYO-SAC DEVELOPMENT IN EUCHLAENA MEXICANA AND ZEA MAYS[1]

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INTRODUCTION

Studies of macrosporogenesis and the development of the macrogametophyte in the Maydeae are almost wholly limited to corn (*Zea mays L.*), and little is known concerning these stages in the life history of other members of this tribe. True (*12*),[3] Guignard (*7*), and Poindexter (*9*) described the ovules of corn, observed double fertilization, and briefly outlined the early stages in the development of the embryo. Weatherwax (*13*, *14*, *15*) and Miller (*8*) studied the details of macrosporogenesis and embryo-sac development. Later Weatherwax (*17*) compared the development of the endosperm of *Coix* (*Coix lacryma-jobi*) with that of corn. More recently Randolph (*10*) has presented an account of the developmental morphology of the caryopsis of maize in which embryogeny, endosperm formation and structure of the pericarp are fully described. The detailed cytological studies of chromosome morphology in teosinte (*Euchlaena mexicana* Schrad.) and teosinte-corn hybrids (Beadle (*2*, *3*); Emerson and Beadle (*6*); Arnason (*1*)), as well as the genetical analyses of such hybrids, warrant a comparative study of macrosporogenesis and embryo-sac development in the two parents and the hybrid.

MATERIALS AND METHODS

The annual type of Florida teosinte was used in the present investigation. This variety, at the latitude of Madison, Wis. (43°), does not come into flower under field conditions until late in the fall. In order to force the plants into flower so as to make the desired crosses with corn, the seeds were planted in 8-inch pots in the greenhouse about the middle of April. The pots were taken to the field about May 15, the precise date depending upon the weather, and sunk into the ground so that the tops of the pots were flush with the level of the soil. A short-day treatment such as was described by Emerson (*5*) was begun at this time. This was accomplished by placing barrels over the plants at 5 o'clock each afternoon and removing them at 7 o'clock the next morning. This practice was continued until the plants began to blossom (about July 15), when the barrels were removed entirely. Plants treated in this manner flowered abundantly and set a good crop of seed. Reciprocal hybrids between yellow dent corn and teosinte were obtained.

Pistillate spikelets of teosinte and of corn of various ages were collected during the summer of 1934, dipped in Carnoy's fluid for a

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[2] The writer desires to express his appreciation for support received from the Wisconsin alumni research fund during the period of these investigations.

[3] Reference is made by number (italic) to Literature Cited, p. 550.

short time (one-half to 1 minute), and transferred directly to Karpechenko's modification of Nawaschin's fluid. The material was allowed to remain in the latter fluid from 36 to 48 hours, then washed, dehydrated, and embedded in 52° paraffin in the usual manner, cedar oil being used as the clearing agent. After embedding, longitudinal sections were cut at thicknesses varying from 12μ to 20μ. The older material was cut thicker in order to obtain the embryo sac in as few sections as possible. The sections were mounted serially, stained in dilute Delafield's haematoxylin, and counterstained in safranine. This combination is valuable because of the clear differentiation of the cytoplasm, spindle, and forming cell plate.

OBSERVATIONS

EUCHLAENA MEXICANA

Development of the Ovule

The single ovule first appears as an erect, rounded protuberance at the base of the carpel, later becoming more or less conical in shape. An apical hypodermal cell becomes differentiated as the primary archesporial cell while the ovule is in an upright position. This cell (fig. 1, *A*, *a*) differs from its neighbors in being conspicuously larger, with a somewhat denser cytoplasm and a larger nucleus (fig. 1, *A*). Shortly after the differentiation of the primary archesporial cell the integuments develop as outgrowths of the epidermis near the base of the ovule on the side away from the central axis of the pistillate spike. The inner integument (*i*) is the first to appear and shortly after its initiation and just below it the primordium of the outer integument starts development. The entire ovule grows more rapidly on the side on which the integuments first appear so that it bends toward the main axis of the spike (fig. 1, *D*). The bending takes place in the region of the origin of the integuments and continues until the mature ovule assumes an amphianatropous position, i. e., it is a type intermediate between the amphitropous and the anatropous forms but approaches the latter (fig. 2, *L*).

The inner integument, which remains two layers of cells in thickness, except at the apex in the mature stages, grows so that it has reached a level even with the tip of the ovule by the time the four spores are formed as a result of macrosporogenesis (fig. 2, *E*). The ovule at this time is so bent that the longitudinal axis of the row of spores makes an angle of approximately 45° to the pedicel. The integument continues to elongate, growing slightly beyond the apex of the nucellus and forming a very short micropyle (fig. 2, *L*). The apical portion in the region of the micropyle becomes four or five layers of cells in thickness by the time the ovule is mature. The outer integument likewise remains two layers of cells in thickness except at the apex, where it may be three or four cells in thickness. It does not develop sufficiently to cover more than two-thirds of the ovule.

The archesporial cell becomes the macrospore mother cell without further division and remains adjacent to the epidermal layer of the ovule until the nucleus shows advancing stages of the meiotic prophases (fig. 1, *B*, C). At later stages the cells of the epidermis divide to form three or four layers of nucellar tissue beyond the spore mother cell (fig. 1, *D*, *E*, *F*).

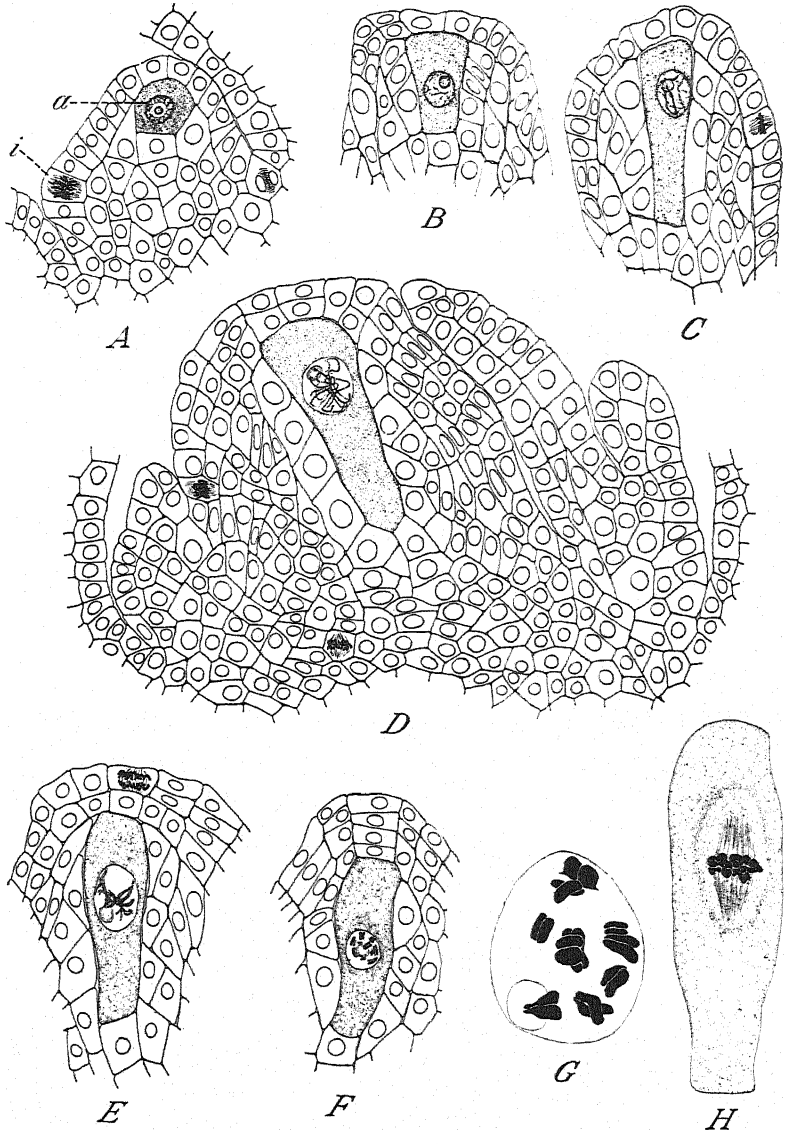


FIGURE 1.—*Euchlaena mexicana: A*, Young ovule; *a*, archesporial cell; *i*, first evidence of integument formation; × 425. *B*, Portion of nucellus with developing archesporial cell; × 425. *C*, Archesporial cell or macrospore mother cell at the onset of meiosis, × 425. *D*, Young ovule with developing integuments. Nucleus of macrospore mother cell at a late spireme stage, × 425. *E*, Portion of nucellus with macrospore mother cell. Nucleus at diplonema, × 425. *F*, Same as *E*, nucleus at diakinesis; × 425. *G*, Nucleus of macrospore mother cell, diakinesis; × 1,625. *H*, Macrospore mother cell, heterotypic equatorial plate stage; × 850. (Drawings made with camera lucida at table level.

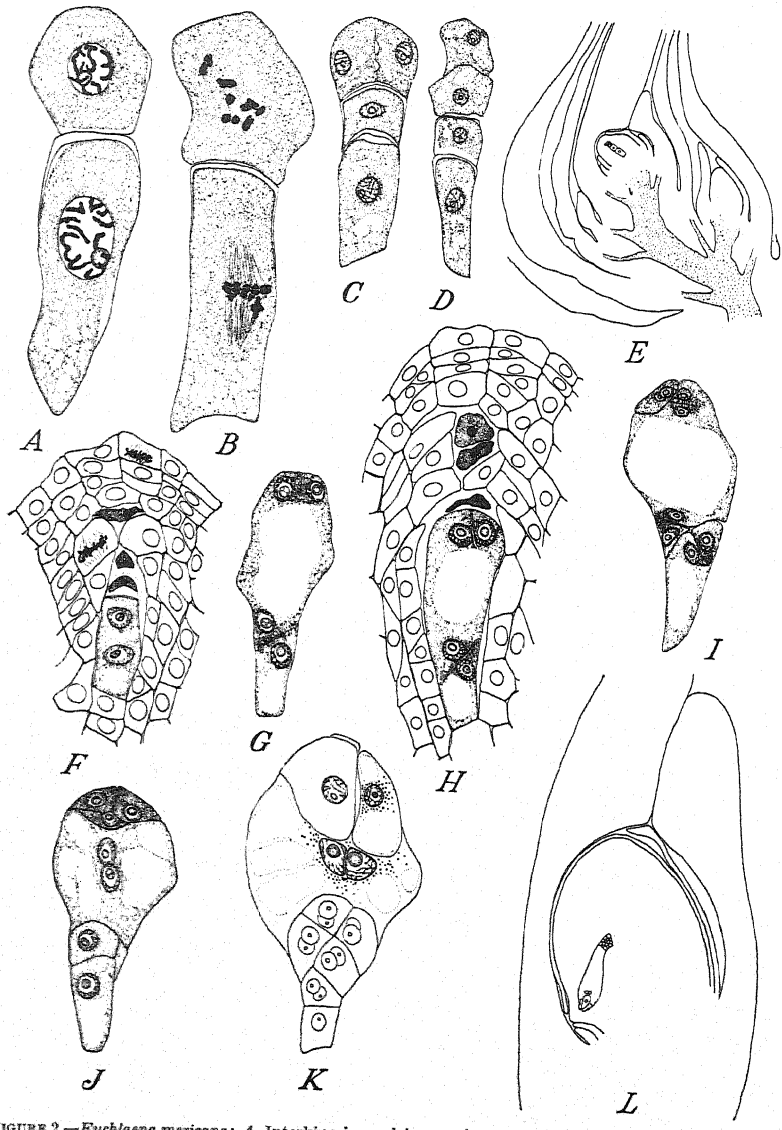


FIGURE 2.—*Euchlaena mexicana: A*, Interkinesis, nuclei preparing to divide; × 850. *B*, Homoeotypic division; × 850. *C* and *D*, Tetrads showing arrangement of spores; × 425. *E*, Longitudinal section through pistillate flower, ovule contains a tetrad of spores; × 45. *F*, Portion of ovule with two-nucleate embryo sac and 3 disintegrating spores; × 425. *G*, Four-nucleate embryo sac, cell-plate formation; × 425. *H*, Portion of ovule with four-nucleate embryo sac, later stage of cell-plate formation; × 425. *I*, Three-celled, eight-nucleate embryo sac showing cell-plate formation; × 425. *J*, Seven-celled, eight-nucleate embryo sac; × 425. *K*, Embryo sac showing increase of antipodal cells and the beginning of starch formation; × 425. *L*, Ovule with maturing embryo sac; shows development of integuments; × 45. (Drawings made with camera lucida at table level.)

Macrosporogenesis

The macrospore mother cell is about twice as long as wide at the onset of meiosis (fig. 1, *B*, *C*). It is somewhat flattened at both ends and wider in the apical portion than at the base. The nucleus, which may be either near the apical end of the cell or in the midregion, passes through the phases characteristic of the heterotypic division (fig. 1, *C* to *H*). Ten pairs of chromosomes are present at diakinesis (fig. 1, *F*), one of which is closely associated with the nucleolus (fig. 1, *G*). The chromosomes at this stage vary in length so that the longest pair is approximately twice as long as the shortest. The spore mother cell increases in size during the heterotypic prophases so that it is fully three times as long as wide at diakinesis.

The cytoplasm of the spore mother cell remains finely vacuolate while the nucleus is passing through the prophases. Toward the end of diakinesis, however, the cytoplasm at the ends of the cell becomes more vacuolate and a denser zone appears immediately surrounding the nucleus (fig. 1, *F*). The heterotypic spindle, whose axis is parallel to the longitudinal axis of the cell, is surrounded by a dense layer of cytoplasm (fig. 1, *H*). At the conclusion of the heterotypic division the spore mother cell is unequally divided by a cell plate, the apical (micropylar) daughter cell being about half as long as the basal (chalazal) cell. A thick wall is laid down by the chalazal cell at its micropylar end during the interim between the two meiotic divisions. The micropylar cell remains thin-walled (fig. 2, *A*).

The axis of the homoeotypic spindle in the chalazal daughter is cell longitudinal; the dense layer of cytoplasm immediately surrounding the spindle is not as apparent as in the preceding division (fig. 2, *B*). This cell likewise divides unequally, and a thick wall is formed on the micropylar end of the basal cell (fig. 2, *C*, *D*). The axis of the spindle in the micropylar cell formed as a result of the first meiotic division may be either longitudinal, transverse, or at an oblique angle to the long axis of the ovule. The nuclear division in this cell lags somewhat behind that in the chalazal cell (fig. 2, *B*). Two thin-walled daughter cells of approximately equal size are formed (fig. 2, *C*, *D*). The four daughter cells resulting from the meiotic divisions thus consist of one elongate chalazal cell which becomes the functional spore and three cells nearly equal in size which ultimately disintegrate. Not more than one spore tetrad was observed in any ovule (fig. 2, *E*).

Development of the Embryo Sac

The cytoplasm of the functional macrospore becomes highly vacuolate toward the chalazal end (fig. 2, *C*, *D*), and ultimately a large vacuole is formed in this region. Shortly after the first nuclear division in the formation of the gametophyte a second vacuole is formed between the two nuclei (fig. 2, *F*). This vacuole becomes much larger than the first, and as a result the nuclei are widely separated. Both daughter nuclei now divide and a distinct cell plate is formed across each spindle (fig. 2, *G*). The embryo sac is thus divided into three regions, namely, a large central region containing two nuclei (one daughter nucleus from each pair) and a large central vacuole (fig. 2, *H*); a chalazal region containing one nucleus and a basal vacuole; and an apical region with one nucleus and finely vacuolate cytoplasm.

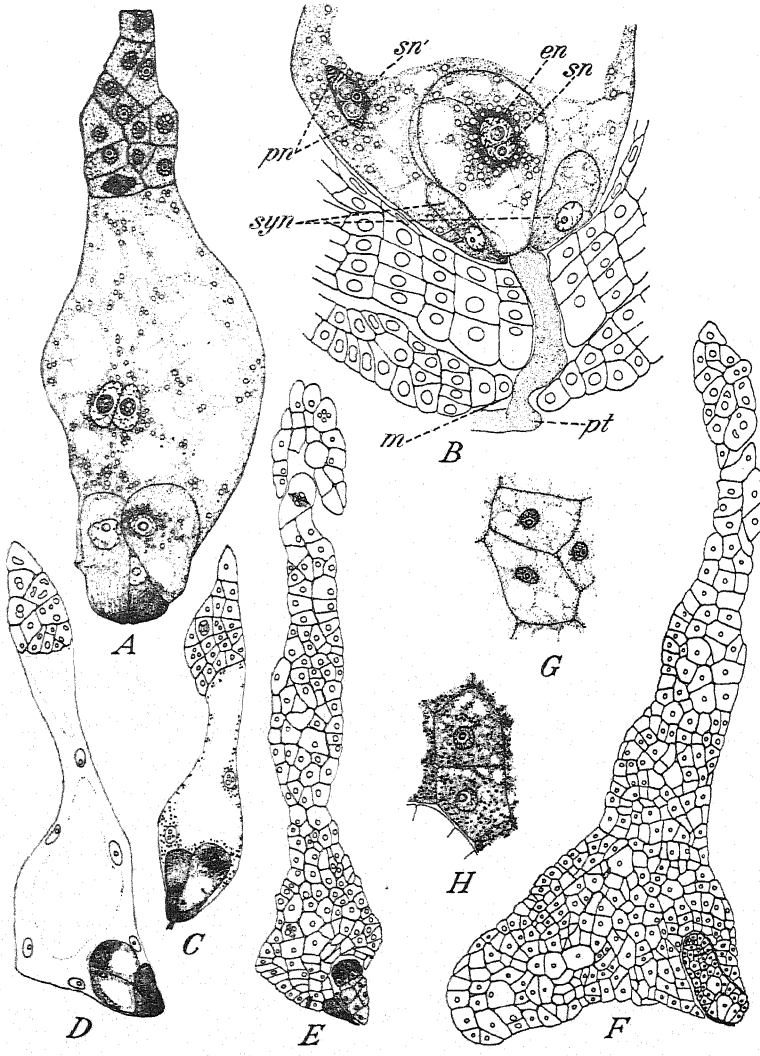


FIGURE 3.—*Euchlaena mexicana: A*, Embryo sac showing starch in egg and endosperm mother cell; × 425. *B*, Apical portion of embryo sac at time of fertilization; tip of pollen tube (*pt*) lies beneath fertilized egg; *pn*, polar nuclei fusing; *sn'*, male gamete nucleus fusing with polar nuclei; *en*, egg nucleus; *sn*, male gamete nucleus fusing with egg nucleus; *syn*, synergids; *m*, micropyle; × 425. *C*, Embryo sac shortly after fertilization showing zygote and two-nucleate endosperm; × 175. *D*, Embryo sac with a two-celled embryo and accompanying suspensor cell. A multinucleate endosperm is present; × 175. *E*, Longitudinal section through endosperm and young embryo with multicellular suspensor(*s*). Note persistent antipodals and synergids; × 175. *F*, Same as *E*. Later stage showing development of endosperm; × 175. *G*, A few endosperm cells from the antipodal region. Highly vacuolate cytoplasm with little evidence of storage materials; × 425. *H*, A few cells of the endosperm lying adjacent to the embryo showing dense cytoplasm and abundant storage materials; × 425. (Drawings made with camera lucida at table level.)

The cell plates formed across the spindles of the second division become increasingly distinct as additional cell plates are formed across the spindles of the third division (fig. 2, *I*). The apical region delimited by the second division divides to form the two synergids. The basal region divides to form two antipodals, one in which the cytoplasm is finely vacuolate and one having a large basal vacuole. The nucleus in the apical portion of the central region divides and a cell plate is formed, cutting off an egg with dense cytoplasm and leaving one nucleus in the central region. Thus the egg nucleus is the sister of one of the polar nuclei. The basal nucleus of the central region likewise divides, and a cell plate cuts off a third antipodal cell, leaving one daughter nucleus in the central region. The large middle cell now contains two nuclei separated by a central vacuole. This vacuole soon disintegrates into a number of smaller ones and the two polar nuclei move to the midregion of the cell, ultimately coming to lie closely adjacent in the vicinity of the egg apparatus (fig. 2, *J*). These nuclei are embedded in a central strand of cytoplasm that extends from the antipodals to the apex of the cell. The seven-celled, eight-nucleate embryo sac now present is approximately three times as long as, and correspondingly wider than, the functional macrospore before the division of its nucleus.

The embryo sac grows to twice its original length (fig. 3, *A*). During its period of growth the antipodal cells divide, reaching a number of 30 or more. These cells have distinct walls and are usually uninucleate, but occasionally a cell with two to four nuclei is present. The nuclei stain heavily and the cytoplasm is finely vacuolate.

The egg apparatus consists of three cells, the egg and two synergids. All of these are somewhat pear-shaped, the smaller ends extending toward the micropyle. The cells increase greatly in size during the final growth period of the embryo sac. The nucleus of each synergid lies in the midregion of the cell just above a large basal vacuole. The apical cytoplasm stains heavily and a conspicuous filiform apparatus is present at the micropylar end of each synergid (fig. 3, *A*).

The egg nucleus is embedded in dense cytoplasm in the midportion of the cell. The cytoplasm except in the vicinity of the nucleus is vacuolate, and a large vacuole occupies the micropylar end of the egg. The primary endosperm cell of the embryo sac remains binucleate. In its cytoplasm, as the cell matures, many small and several large vacuoles are formed. The two polar nuclei, lying in the apical portion of the cell, are in close contact. Many starch grains are present both in this cell and in the egg; there appear to be none in the synergids and antipodals. No starch is to be seen in the newly formed seven-celled embryo sac (fig. 2, *J*). As the sac develops, however, starch grains are formed in the dense cytoplasm immediately surrounding the polar nuclei and in that about the egg nucleus (fig. 2, *K*). The egg enlarges greatly just prior to fertilization, so that it extends into the embryo sac for some distance beyond the basal ends of the synergids.

Fertilization and Development of the Embryo

Fertilization takes place between 15 and 20 hours after pollination. The pollen tube enters the embryo sac between the synergids; usually neither of these is disorganized at this time (fig. 3, *B*, *C*). One of the

male nuclei (*sn*) fuses with the egg nucleus (*en*), and the other (*sn'*) fuses with the two polar nuclei (*pn*). Both fusions are shown in figure 3, *B*. The end of the pollen tube (*pt*) can be seen in the micropyle and extending between the apical cells of the nucellus. Its rounded tip lies beneath the zygote. The tube is constricted in the region where it is surrounded by the apices of the synergids and zygote. The dense cytoplasm of the primary endosperm cell becomes reorganized at or shortly after fertilization so that it forms a thin layer lining the periphery, a large vacuole occupying the central region of the cell. It is difficult to analyze the details of the zygote nucleus because of the many starch grains present in the immediately adjacent dense cytoplasm. Much starch is present also in the endosperm cytoplasm, especially in the region of the fusing nuclei. Division of the endosperm nucleus occurs almost immediately after fertilization; two and often four nuclei are formed before the complete disintegration of the remnants of the pollen tube.

Four to eight nuclei are present in the endosperm before the zygote divides; by the time a four-celled embryo is formed 25 to 30 endosperm nuclei lie in the thin peripheral layer of dense cytoplasm (fig. 3, *D*). Soon cell walls are formed between the endosperm nuclei, and large highly vacuolate cells are formed. Those endosperm cells in the neighborhood of the embryo now divide rapidly so that many small cells are present in this region, whereas the endosperm cells in the region of the antipodals are large and highly vacuolate. The endosperm contains many cells by the time the embryo reaches the stage shown in figure 3, *E*, in which the suspensor (*s*) is well differentiated. The endosperm develops at the expense of the nucellus, which in time is completely destroyed with the exception of the epidermis, which becomes a part of the seed coat. From this time on growth of the embryo is particularly active in two directions, toward the antipodals, which form a basal cap, and toward the nucellus at a right angle to the longitudinal axis of the embryo.

In material collected 5 days after pollination, the outgrowth of the endosperm directly opposite the embryo is more pronounced and the whole mass of endosperm has greatly increased in size. The peripheral layer of endosperm cells at this stage is conspicuous because of a differential staining reaction. Very little storage material is observed in the endosperm (fig. 3, *A*), except in those cells immediately adjacent to the embryo. The cytoplasm of these cells is dense and stains heavily and in some cells starch grains are present (fig. 3, *H*). The synergids are usually both intact although somewhat shrunken. They lie immediately beneath the suspensor shown in figure 3, *F*. The antipodal cells are well formed at this stage and show no signs of disintegration.

ZEA MAYS

The ovule of corn develops in a manner similar to that described for teosinte. The form at maturity is likewise similar. A hypodermal cell in the apical region becomes the primary archesporial cell (fig. 4, *A*). This cell does not divide to form a primary parietal and primary sporogenous cell but functions as a macrospore mother cell. The longitudinal axis of the heterotypic spindle is parallel with the long axis of the cell (fig. 4, *B*). After the first meiotic division two unequal daughter cells are formed, the chalazal cell being about twice

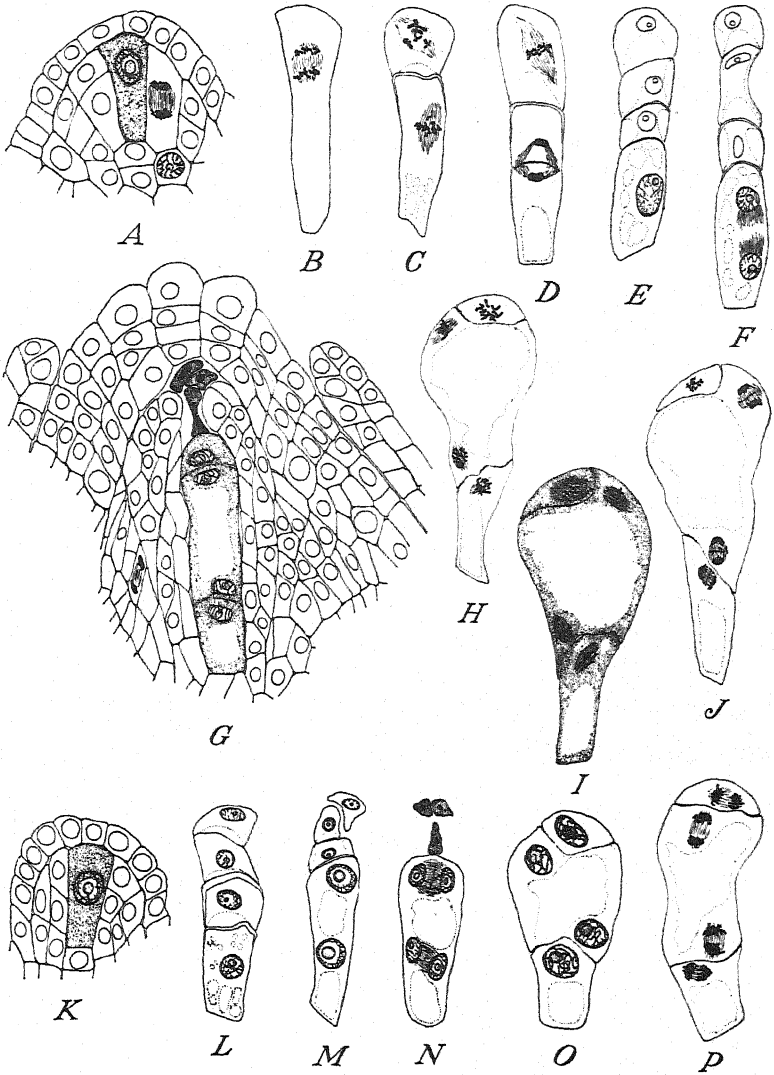


FIGURE 4.—*Zea mays: A*, Apical portion of ovule showing archesporial cell and dividing nucellar cells. *B*, Macrospore mother cell, heterotypic anaphase. *C*, Homoeotypic division, equatorial plate in basal cell and an earlier stage of division in apical cell. *D*, Same as *C*. Cell-plate formation in basal cell and equatorial plate in apical cell. *E*, Tetrad of spores. Large basal cell is the functional spore. *F*, Two-nucleate embryo sac with three disintegrating spores. *G*, Apical portion of ovule showing a four-nucleate, three-celled embryo sac and three disintegrating spores. *H*, Third division in embryo sac. Chromosomes advancing to the equatorial plates. *I*, Sames as *H*. Equatorial plate stage. *J*, Same as *H*. Telophase showing cell-plate formation. All × 425.

Zea mays × *Euchlaena mexicana: K*, Apical portion of ovule with archesporial cell. *L*, Tetrad of spores. *M*, Two-nucleate embryo sac and three disintegrating spores. *N*, Four-nucleate embryo sac showing cell-plate formation. *O*, Four-nucleate, three-celled embryo sac. *P*, Third division in embryo sac, telophase. All × 425. (Drawings made with camera lucida at table level.)

as long as the micropylar one. A thick wall is formed at the apex of the chalazal cell immediately after this division.

During the homoeotypic division the longitudinal axis of the spindle in the chalazal cell is approximately parallel to the long axis of the cell, whereas the axis of the spindle in the micropylar cell is usually at an angle (fig. 4, *C*, *D*). Nuclear and cell division in the chalazal cell usually precede division in the micropylar cell. The chalazal cell divides unequally and the innermost is the larger of the two daughter cells (fig. 4, *E*). A thick wall is formed at the apex of the innermost cell. The micropylar cell divides so that the two daughter cells are approximately equal in size. Of the four spores now present, the innermost and largest becomes the functional spore (fig. 4, *E*). The other three, approximately equal in size, ultimately disintegrate.

The nucleus of the functional macrospore divides (fig. 4, *F*). The daughter nuclei pass to opposite ends of the sac and then divide. Cell plates are laid down across the spindles of this second division in such a way that a three-celled embryo sac is formed consisting of a uninucleate cell at each end and a large central binucleate cell (fig. 4, *G*). By the time the embryo sac has reached this stage the three nonfunctional spores are in an advanced stage of disintegration.

The embryo sac is fully twice as long at the time of the third division as was the functional macrospore. The sac has likewise increased in diameter in the micropylar region, the diameter here being three times that of the spores. In consequence, the embryo sac is somewhat pear-shaped at the time of the third division (fig. 4, *H*, *I*, *J*). Cell plates are laid down across the spindles of the third division (fig. 4, *J*), in a manner similar to that described for teosinte, a seven-celled, eight-nucleate embryo sac being formed. The egg nucleus is a sister of one of the polar nuclei. Later the antipodal cells increase in number (30 to 40 or more) by division and a large amount of starch is stored in the egg and the primary endosperm cell. Many of the antipodal cells contain two or more nuclei each. This antipodal tissue remains as a well-developed structure in almost mature grains of corn which were collected 25 days after pollination.

ZEA MAYS × EUCHLAENA MEXICANA

The process of macrosporogenesis in *Zea mays* × *Euchlaena mexicana* is essentially similar to that found in both parents. The primary archesporial cell becomes the spore mother cell. (fig. 4, *K*). Four spores are formed as the result of the two meiotic divisions, the two toward the chalaza having thick walls at their micropylar ends (fig. 4, *L*). The innermost and largest spore becomes the macrospore and the others disintegrate. The developing macrogametophyte passes through stages similar to those described for teosinte (fig. 4, *M* to *P*). Conspicuous cell plates form across the spindles of the second division (fig. 4, *N*). The sac is now three-celled and four-nucleate (fig. 4, *O*). All four nuclei divide (fig. 4, *P*), cell-plate formation ensues, and an eight-nucleate, seven-celled embryo sac is the result. The antipodal cells divide so that 30 to 40 are present in the mature gametophyte. Starch grains are formed in the egg and in the primary endosperm cell. The starch is particularly abundant in the neighborhood of the egg nucleus and of the fusion nuclei.

DISCUSSION

The ovules of teosinte, corn, and the hybrid between them are amphianatropous at the time of maturity. True (*12*) described the ovule of corn as being campylotropous; Miller (*8*) and Randolph (*10*) found it to be of a modified campylotropous type; Weatherwax (*13*) reported it as anatropous. However, his figure of the ovule of *Coix* shortly after fertilization is similar to the ovules of *Euchlaena* and *Zea* as herein described.

The primary archesporial cell in the three forms studied functions directly as the sporogenous cell without further division. Weatherwax (*15*) described a periclinal division of the archesporial cell of corn forming a parietal cell and the macrospore mother cell. No cell wall is formed, however, according to him, and the parietal cell is "immediately consumed." The writer has examined a large number of preparations containing many stages in the development of the archesporial cell in order to find evidences of such a division. During the early stages of development of the ovule the nucellar cells adjacent to the archesporial cell divide periclinally in the manner shown in figure 4, *A*, but no evidence of a division of the archesporial cell was observed prior to the first meiotic division.

The spore mother cell divides to form four spores. The thick walls at the micropylar ends of the two innermost of the four spores are particularly conspicuous in teosinte and in the corn-teosinte hybrid. These walls take a deep purple color when stained with Delafield's haematoxylin and a bright pink color when the iodine-gentian violet combination is used. In no case observed were only three spores formed because of a failure of the completion of the homoeotypic division in the micropylar cell as described by Weatherwax (*15*).

The chalazal spore functions as a macrospore and the three apical spores disintegrate. Miller (*8*) reported that all four spores enter into the formation of the embryo sac. On the other hand, Weatherwax (*15*) found, as the writer has, that the chalazal spore alone continues development and the other three become disorganized. Brink (*4*), in a plant heterozygous for the waxy type of starch, found approximately a 1–1 segregation of the two types of starch in the embryo sac. This evidence excludes the possibility of all four macrospores entering into the formation of a macrogametophyte.

The development of the embryo sac is interesting because of the cell-plate formation across the spindles of the second division. In the material stained with Delafield's haematoxylin the plates are clearly differentiated. This fact, in addition to the position of the spindles of the third division, substantiates the observation of Weatherwax that one of the polar nuclei is a sister of the egg nucleus. Figure 2, *I*, showing cell-plate formation after the third nuclear division in teosinte, shows that the synergids on the one hand, and the egg nucleus and the upper polar nucleus on the other, represent sister nuclei. Schnarf (*11*) considers this as probably being the case in most, if not all angiosperms.

During the period of further maturation of the macrogametophyte the antipodal cells increase in number by division until 30 to 40 or more are present. These cells are of approximately the same size in teosinte and corn. Those of *Coix* (*17*) are fewer in number, much larger, and more highly vacuolate. This antipodal tissue remains

intact for a long period after fertilization, forming a cap at the apex of the endosperm. Material of teosinte 5 days after pollination shows well-developed antopodal cells as do also corn kernels collected 25 days after pollination. Weatherwax (*16*) and Randolph (*10*) found evidence of the presence of antipodal tissue just opposite the dent in fully matured corn kernels.

SUMMARY

Ovule development, macrosporogenesis, and the formation of the macrogametophyte are essentially similar in the annual variety of Florida teosinte, yellow dent corn, and a corn-teosinte hybrid.

Just before the appearance of the primordia of the integuments, a single hypodermal cell of the nucellus is differentiated as a primary archesporial cell. This cell functions as a macrospore mother cell.

The macrospore mother cell by two divisions produces a row of four macrospores.

The chalazal spore becomes the embryo-sac mother cell and the three micropylar spores disintegrate.

After the second nuclear division in the embryo sac, cell plates are formed across the spindles, producing a three-celled embryo sac.

A third nuclear division, followed by cell division leads to the formation of an eight-nucleate, seven-celled embryo sac. The two synergid nuclei are sister nuclei; so are the egg nucleus and one polar nucleus.

The antipodal cells continue to divide during the course of maturation and growth of the embryo sac so that 30 to 40 cells or more are formed. These persist as a definite tissue in the developing seed.

During the later stages of the maturation of the embryo sac starch is stored in the egg and the primary endosperm cell.

The ovule at the time of fertilization is amphianatropous in form.

Fertilization occurs in teosinte between 15 and 20 hours after pollination.

The synergids are not disorganized as a result of the entrance of the pollen tube. They persist in the region of the micropyle for 4 to 5 days and then disintegrate.

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DISTRIBUTION AND VARIATION WITH MATURITY OF DISSOLVED SOLIDS, SUCROSE, AND TITRATABLE ACIDITY IN THE SORGO STALK[1]

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EARLIER WORK

Joulie [2] in his earlier work on sorgo as a source of sugar reported the relative weights of the internodes and the ash and sugar content of the juices expressed from them separately. Subsequent investigators ascribed various qualities to the juices expressed from the upper and lower portions of the sorgo stalk, and made widely varying recommendations for topping sorgo for sirup or sugar manufacture.

During the latter half of the nineteenth century, when sorgo was extensively investigated as a commercial source of sugar, there was some confusion regarding the part of the stalk best suited for commercial utilization. Studies made at that time were largely on gross sections of the sorgo stalk, such as thirds and halves, instead of internode divisions. Collier [3] concluded that there was no marked difference in the quantity or quality of the juices from the upper and lower half of the sorgo stalk. He based his conclusions on determinations of specific gravity and total amount of dissolved solids in the juice. Wiley [4] concluded from analyses of thirds of the stalk that at full maturity there was practically no difference in the juice from the upper and lower halves of the sorgo stalk. On the other hand, he [5] stated that studies of Amber cane in Italy seemed to show that the upper internodes and the peripheric strata contained a greater abundance of saccharose than the lower internodes and the central strata.

Martin, Harris, and Jones,[6] in a study of the freezing-point depression and specific conductivity of sorghum tissue fluids, found that when sorgo plants were nearly mature, that is, in the "dough-to-ripe" stage, which is the stage of maturity usually considered best for sirup making, the dissolved solids content of the juice was not the same in the different internodes. It increased from the first to the fourth internode; in the fifth and sixth it was about the same as in the fourth.

More recently one of the writers,[7] in a preliminary study of the quality of sirup produced from various portions of the stalk, corrob-

[1] Received for publication May 19, 1937; issued November 1937.

[2] JOULIE, H. ÉTUDES ET EXPÉRIENCES SUR LE SORGHO À SUCRE CONSIDERÉ AU POINT DE VUE BOTANIQUE, AGRICOLE, CHIMIQUE, PHYSIOLOGIQUE ET INDUSTRIEL. 200 pp., illus. Paris. 1864. (See pp. 101, 144, 145.)

[3] COLLIER, P. REPORT OF THE CHEMIST OF THE AGRICULTURE DEPARTMENT TO COMMISSIONER W. G. LE DUC. Letter of the Commissioner of Agriculture to Honorable J. W. Johnston, Chairman, United States Senate Committee on Sorghum Sugar. 42 pp. 1880. (See p. 26.)

[4] WILEY, H. W. DIFFUSION, ITS APPLICATION TO SUGAR-CANE AND RECORD OF EXPERIMENTS WITH SORGHUM IN 1883. U. S. Dept. Agr., Chem. Div. Bull. 2, 36 pp., illus. 1884. (See p. 24.)

[5] ——— THE NORTHERN SUGAR INDUSTRY. A RECORD OF ITS PROGRESS DURING THE SEASON OF 1883. U. S. Dept. Agr., Chem. Div. Bull. 3, 118 pp., illus. 1884. (See p. 79.)

[6] MARTIN, J. H., HARRIS, J. A., and JONES, I. D. FREEZING-POINT DEPRESSION AND SPECIFIC CONDUCTIVITY OF SORGHUM TISSUE FLUIDS. Jour. Agr. Research 42: 57–69. 1931.

[7] VENTRE, E. K. A STUDY OF THE SORGO STALK WITH REFERENCE TO ITS SIRUP MAKING QUALITIES. Presented at Amer. Chem. Soc. meeting, San Francisco, Aug. 9–13, 1935.

orated, in the main, the conclusions of Martin, Harris, and Jones. In considering, therefore, the contradictory results reported by investigators since the time of Joulie, the need for a more detailed study of the sorgo stalk was apparent. It was thought that such a study might form a basis for selection of that portion of the sorgo stalk most suited for efficient commercial utilization, and possibly throw some additional light on the failure of sorgo in the past as a commercial source of sugar.

PRESENT WORK

PROCEDURE

In cooperation with the Mississippi Agricultural Experiment Station, the four varieties of sorgo (*Holcus sorghum* var. *saccharatus*) most used for sirup production in that section were selected for study. The different varieties, designated by the names locally assigned to them, presented the following varietal characteristics:

Hodo.—A large-barrel, long-stalk, late-maturing variety.

Iceberg.—A small-barrel, short-stalk, early-maturing variety.

Honey.—A small-barrel, medium-height stalk, intermediate-maturing, sprangle-top variety.

Gooseneck.—A medium-barrel, medium-height stalk, intermediate-maturing variety.

The stages of maturity selected for study were determined by the average maturity of the seed heads, described as follows:

Milk stage.—When the seed heads were out of the sheath and well formed, and the contents when pressed out had the consistence of milk.

Dough-to-ripe stage.—When the contents pressed from the seed heads had the consistence of stiff dough, the stage just preceding the solid stage.

Dead-ripe stage.—When the contents of the seed heads were solid, and the glumes were wide open and dry.

When the sorgo had reached the selected stage of maturity, the stalks were cut even with the top surface of the row and stripped of leaves. The seed head and peduncle were removed, and the stalks divided at the nodes. The internodes were numbered consecutively from the peduncle and segregated in batches according to number. Each batch was weighed and ground separately in a horse-driven farm mill. Each was passed through the mill twice in order to extract as much juice as possible with this type of milling equipment.

A whole-stalk sample of each variety at each stage of maturity was used for comparison. An aliquot portion of the stalks was selected and stripped clean, and after the seed heads and peduncle were removed, it was subjected to exactly the same treatment as the various internode batches.

The juice expressed from each set of internodes was collected separately and weighed, and the following determinations were made on the expressed juice:

Dissolved solids or degrees Brix.—Determined by a Brix hydrometer calibrated at 20° C.

Sucrose.—Determined polariscopically after defecation with Horne's dry lead.[8]

Titratable acidity.—A 10-ml portion was titrated with 0.1 normal sodium hydroxide. Phenolphthalein was used as an indicator.

[8] SPENCER, G. L., and MEADE, G. P. A HANDBOOK FOR CANE-SUGAR MANUFACTURERS AND THEIR CHEMISTS. Ed. 7, rewritten and enl. by G. P. Meade, 560 pp., illus. New York and London. 1929. (See p. 225.)

DATA

Tables 1 to 4 give the data obtained for the four varieties of sorgo at different stages of maturity.

Column 2 of each table lists the weight of the stalk in each internode batch.

Column 3 lists the percentage of the total weight contributed by each batch of internodes. The figures are obtained by dividing the weight of each batch of internodes (column 2) by the total weight of stalks ground, or the total of column 2.

Column 4 lists the weight of the juice expressed from each batch of internodes.

TABLE 1.—*Yield and composition of juice from various internodes of Gooseneck variety of sorgo at different stages of maturity*

MILK STAGE

Internode no.	Weight		Juice expressed		Dissolved solids in expressed juice, by Brix			Sucrose in expressed juice		Apparent purity of juice	Acidity expressed in ml of N/10 NaOH	
											Per 10 ml juice	Per gram Brix solids in expressed juice
(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)	(12)	(13)
	Lb.	*Pct.*	*Lb.*	*Pct.*	*Pct.*	*Lb.*	*Pct.*[1]	*Pct.*	*Lb.*		*Cc*	*Cc*
1	15.0	3.44	6.0	40.00	12.12	0.73	2.37	1.59	0.09	13.11	3.2	2.51
2	23.0	5.27	12.0	52.17	12.96	1.55	5.03	2.97	.36	22.91	2.5	1.84
3	33.0	7.57	19.0	57.57	13.36	2.54	8.24	3.52	.67	26.30	1.5	1.06
4	41.0	9.40	24.0	58.53	13.30	3.19	10.34	3.64	.87	27.36	1.4	1.00
5	44.0	10.10	24.0	54.54	13.56	3.25	10.54	4.34	1.04	32.00	.8	.56
6	47.0	10.78	26.0	55.31	13.45	3.50	11.35	4.69	1.22	34.86	.7	.49
7	50.0	11.47	28.0	56.00	13.22	3.70	12.00	4.97	1.39	37.59	.7	.50
8	50.0	11.47	28.0	56.00	12.62	3.53	11.44	4.21	1.18	33.35	.7	.53
9	49.0	11.24	28.0	57.14	12.50	3.50	11.35	.89	.25	7.12	.7	.54
10	44.0	10.09	24.0	54.54	11.90	2.85	9.24	.60	.14	5.04	.7	.56
11	31.0	7.11	16.0	51.61	12.00	1.92	6.22	.50	.08	4.16	.8	.64
12	9.0	2.06	5.0	55.55	11.59	.58	1.88	.45	.02	3.88	.8	.66
Total or average	436.0	100.00	240.0	55.04	12.85	30.84	100.0	3.05	7.31	23.70	------	.74

DOUGH-TO-RIPE STAGE

1	16.0	3.15	6.0	37.50	15.88	0.95	2.47	7.09	0.45	44.64	3.9	2.31
2	21.0	4.13	9.0	42.85	17.02	1.53	3.97	7.91	.71	46.47	3.0	1.65
3	28.0	5.51	14.5	51.78	17.22	2.49	6.46	9.26	1.34	53.77	2.2	1.19
4	34.0	6.69	19.0	55.88	16.82	3.19	8.28	8.86	1.68	52.67	1.5	.84
5	42.0	8.27	22.0	52.38	16.02	3.52	9.14	7.63	1.67	47.62	1.5	.88
6	50.0	9.84	27.0	54.00	15.12	4.08	10.59	6.43	1.73	42.52	1.4	.87
7	62.0	12.21	34.0	54.84	14.52	4.93	12.80	5.14	1.74	35.40	1.2	.78
8	69.0	13.58	38.0	55.07	14.22	5.40	14.02	4.49	1.70	31.57	1.1	.73
9	69.0	13.58	34.0	49.27	14.38	4.89	12.69	3.88	1.32	26.98	1.1	.72
10	58.0	11.42	29.0	50.00	14.18	4.11	10.67	3.84	1.11	27.08	1.1	.73
11	39.0	7.68	18.0	46.15	13.78	2.48	6.44	3.60	.65	26.12	1.0	.69
12	20.0	3.94	7.0	35.00	13.58	.95	2.47	3.01	.21	22.16	.9	.63
Total or average	508.0	100.00	257.5	50.68	14.95	38.52	100.00	5.55	14.31	37.15	------	.87
Whole stalk	74.0	------	42.0	56.75	14.46	------	------	7.29	------	50.40	1.6	1.04

[1] Of total.

TABLE 1.—*Yield and composition of juice from various internodes of Gooseneck variety of sorgo at different stages of maturity*—Continued

DEAD-RIPE STAGE

Internode no.	Weight		Juice expressed		Dissolved solids in expressed juice, by Brix			Sucrose in expressed juice		Apparent purity of juice	Acidity expressed in ml of N/10 NaOH	
											Per 10 ml juice	Per gram Brix solids in expressed juice
(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)	(12)	(13)
	Lb.	*Pct.*	*Lb.*	*Pct.*	*Pct.*	*Lb.*	*Pct.*	*Pct.*	*Lb.*		*Cc*	*Cc*
1	15.0	3.31	7.00	46.66	16.81	1.18	3.09	8.73	0.61	51.93	4.4	2.45
2	20.5	4.51	10.25	50.00	17.91	1.83	4.80	10.23	1.05	57.11	3.8	1.98
3	26.5	5.83	13.50	50.94	18.31	2.47	6.48	11.42	1.54	62.37	2.9	1.47
4	32.5	7.15	18.00	55.38	18.31	3.29	8.63	11.52	2.07	62.92	2.5	1.27
5	38.0	8.36	20.00	52.63	18.11	3.62	9.49	10.33	2.07	57.04	2.2	1.13
6	43.5	9.57	22.00	50.57	17.71	3.89	10.20	9.96	2.19	56.23	2.0	1.05
7	51.5	11.33	25.00	48.54	17.01	4.25	11.13	9.11	2.28	53.55	1.9	1.04
8	58.0	12.76	30.00	51.72	16.61	4.98	13.06	8.40	2.52	50.57	1.8	1.01
9	58.5	12.87	29.00	49.57	16.54	4.79	12.56	6.69	1.94	40.45	1.8	1.02
10	54.0	11.88	24.00	44.44	16.44	3.94	10.33	6.44	1.55	39.17	1.7	.97
11	39.0	8.58	17.00	43.58	16.24	2.76	7.24	6.60	1.12	40.64	1.5	.87
12	17.5	3.85	7.00	40.00	16.14	1.13	2.97	6.41	.45	39.71	1.5	.87
Total or average	454.5	100.00	222.75	49.00	17.11	38.13	100.00	8.70	19.39	50.85	------	1.15
Whole stalk	73.5	------	42.00	57.14	15.87	------	------	8.95	------	56.39	2.1	1.24

TABLE 2.—*Yield and composition of juice from various internodes of Honey variety of sorgo at different stages of maturity*

MILK STAGE

Internode no.	Weight		Juice expressed		Dissolved solids in expressed juice, by Brix			Sucrose in expressed juice		Apparent purity of juice	Acidity expressed in ml of N/10 NaOH	
											Per 10 ml juice	Per gram Brix solids in expressed juice
(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)	(12)	(13)
	Lb.	*Pct.*	*Lb.*	*Pct.*	*Pct.*	*Lb.*	*Pct.*	*Pct.*	*Lb.*		*Cc*	*Cc*
1	23.0	3.39	10.0	43.48	12.56	1.25	2.84	2.91	0.29	23.17	3.3	2.50
2	28.0	4.13	14.0	50.00	13.59	1.90	4.31	4.00	.56	29.43	2.1	1.46
3	36.0	5.31	20.0	55.55	14.19	2.84	6.46	4.95	.99	34.88	1.7	1.13
4	48.0	7.09	28.0	58.33	14.19	3.97	9.02	4.73	1.32	33.33	1.0	.67
5	61.0	9.00	35.0	57.37	13.19	4.61	10.48	3.76	1.32	28.50	1.0	.72
6	70.3	10.33	41.0	58.57	12.59	5.16	11.73	3.37	1.38	26.77	.9	.68
7	79.0	11.66	45.0	56.96	12.29	5.53	12.57	3.00	1.35	24.41	.8	.62
8	90.0	13.29	46.0	51.11	11.79	5.42	12.32	2.53	1.16	21.45	.8	.65
9	86.0	12.69	43.0	50.00	11.59	4.98	11.32	.57	.25	4.92	.8	.66
10	79.0	11.66	37.0	46.83	11.59	4.29	9.75	.65	.24	5.60	.8	.66
11	52.0	7.68	24.0	46.15	11.49	2.76	6.27	.67	.16	5.83	.8	.66
12	25.5	3.77	11.0	43.14	11.71	1.29	2.93	.87	.09	7.43	.8	.65
Total or average	677.5	100.00	354.0	52.25	12.43	44.00	100.00	2.57	9.11	20.68	------	.78
Whole stalk	79.0	------	44.0	55.69	12.22	------	------	2.63	------	21.52	1.2	.93

TABLE 2.—*Yield and composition of juice from various internodes of Honey variety of sorgo at different stages of maturity*—Continued

DOUGH-TO-RIPE STAGE

Internode no.	Weight		Juice expressed		Dissolved solids in expressed juice, by Brix			Sucrose in expressed juice		Apparent purity of juice	Acidity expressed in ml of N/10 NaOH	
											Per 10 ml juice	Per gram Brix solids in expressed juice
(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)	(12)	(13)
	Lb.	*Pct.*	*Lb.*	*Pct.*	*Pct.*	*Lb.*	*Pct.*	*Pct.*	*Lb.*		*Cc*	*Cc*
1	24.5	3.47	10.75	43.87	15.74	1.69	3.15	7.26	0.78	46.12	3.9	2.33
2	28.0	3.96	12.50	44.64	17.34	2.16	4.03	9.50	1.18	54.78	2.7	1.45
3	34.0	4.81	16.50	48.52	18.04	2.97	5.53	10.52	1.73	58.31	1.6	.82
4	45.5	6.44	23.50	51.64	18.74	4.40	8.20	10.56	2.48	56.35	1.1	.55
5	60.0	8.49	33.00	55.00	17.87	5.89	10.98	9.53	3.14	53.32	1.0	.52
6	70.0	9.91	37.50	53.57	17.07	6.40	11.93	8.57	3.21	50.20	.9	.49
7	82.0	11.61	41.00	50.00	16.47	6.75	12.58	7.43	3.04	45.11	.9	.51
8	89.0	12.60	46.00	51.68	15.87	7.30	13.60	6.26	2.88	39.44	.9	.53
9	90.0	12.74	39.00	43.33	15.24	5.94	11.07	4.85	1.89	31.82	.9	.55
10	90.0	12.74	37.00	41.11	14.84	5.49	10.23	3.93	1.45	26.48	.9	.57
11	68.0	9.62	24.50	36.03	14.76	3.61	6.73	2.70	.66	18.29	.9	.57
12	25.5	3.61	7.25	28.43	14.66	1.06	1.97	2.12	.15	14.46	.9	.58
Total or average	706.5	100.00	328.50	46.49	16.33	53.66	100.00	6.88	22.59	42.13	------	.64
Whole stalk	70.0	------	34.50	49.29	15.64	------	------	6.43	------	41.11	1.5	.90

DEAD-RIPE STAGE

1	22.5	3.11	9.5	42.22	14.50	1.37	2.03	3.56	0.35	24.55	4.3	2.80
2	26.0	3.60	14.0	53.84	16.40	2.69	3.98	5.42	.76	33.04	3.0	1.71
3	43.5	6.02	18.5	42.52	17.30	3.20	4.74	6.81	1.26	39.36	2.1	1.13
4	45.5	6.29	26.5	58.24	18.10	4.79	7.09	8.39	2.22	46.35	1.8	.92
5	58.0	8.02	36.0	62.06	19.08	6.87	10.18	4.83	1.74	25.31	1.8	.87
6	71.0	9.82	40.5	57.04	18.88	7.64	11.32	5.95	2.40	31.51	1.5	.73
7	79.0	10.93	46.0	58.22	18.36	8.44	12.50	5.42	2.49	29.52	1.2	.61
8	91.5	12.65	50.0	54.64	17.98	8.99	13.32	4.03	2.01	22.41	1.2	.62
9	95.0	13.14	47.5	50.00	17.87	8.49	12.57	2.92	1.38	16.34	1.2	.62
10	97.0	13.42	45.0	46.39	17.67	7.95	11.78	1.32	.59	7.47	1.2	.63
11	67.0	9.27	29.0	43.28	18.17	5.23	7.75	1.79	.52	9.85	1.0	.51
12	27.0	3.73	10.0	37.03	18.57	1.85	2.74	.88	.08	4.73	1.0	.50
Total or average	723.0	100.00	372.5	51.52	18.12	67.51	100.00	4.14	15.80	23.40	------	.78
Whole stalk	70.5	------	40.5	57.44	16.26	------	------	6.95	------	42.74	1.4	.80

TABLE 3.—*Yield and composition of juice from various internodes of Iceberg variety of sorgo at different stages of maturity*

DOUGH-TO-RIPE STAGE

Internode no.	Weight		Juice expressed		Dissolved solids in expressed juice, by Brix			Sucrose in expressed juice		Apparent purity of juice	Acidity expressed in ml of N/10 NaOH	
											Per 10 ml juice	Per gram Brix solids in expressed juice
(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)	(12)	(13)
	Lb.	*Pct.*	*Lb.*	*Pct.*	*Pct.*	*Lb.*	*Pct.*[1]	*Pct.*	*Lb.*		*Cc*	*Cc*
1	21.5	4.86	12.0	65.53	18.95	2.27	4.60	11.98	1.44	63.21	2.7	1.32
2	29.0	6.56	18.0	62.06	19.25	3.46	7.01	12.88	2.31	66.90	2.4	1.15
3	36.0	8.14	24.0	66.66	19.55	4.69	9.50	13.50	3.24	69.05	1.6	.76
4	44.0	9.94	30.0	68.18	19.63	5.89	11.94	13.35	4.00	68.00	1.5	.71
5	49.0	11.07	32.0	65.30	18.95	6.06	12.28	12.13	3.88	64.01	1.4	.68
6	55.0	12.43	35.0	63.63	18.45	6.45	13.07	11.52	4.03	62.43	1.2	.60
7	59.0	13.33	32.0	54.23	17.85	5.71	11.57	10.62	3.39	59.49	1.1	.57
8	59.0	13.33	35.0	59.32	17.55	6.14	12.44	9.77	3.42	55.66	1.0	.53
9	54.0	12.20	30.0	55.55	17.19	5.16	10.46	8.22	2.46	47.81	1.0	.54
10	36.0	8.14	21.0	58.33	16.79	3.52	7.13	7.06	1.48	42.04	1.0	.55
Total or average	442.5	100.0	269.0	60.79	18.34	49.35	100.0	11.02	29.65	60.08	------	.68
Whole stalk	124.0	------	78.0	62.90	18.25	------	------	11.59	------	63.50	1.5	.76

DEAD-RIPE STAGE

1	18.5	4.86	8.25	44.59	21.44	1.77	4.41	14.58	1.20	68.00	5.5	2.36
2	23.5	6.17	11.25	47.87	22.44	2.52	6.28	16.04	1.80	71.42	4.5	1.83
3	29.0	7.61	15.50	53.44	22.84	3.54	8.83	16.68	2.58	73.02	3.3	1.32
4	37.0	9.71	21.00	56.75	22.44	4.71	11.75	17.03	3.57	75.89	2.7	1.10
5	46.0	12.07	23.00	50.00	22.24	5.11	12.74	15.56	3.58	70.00	2.5	1.03
6	48.0	12.60	24.00	50.00	21.84	5.24	13.07	15.03	3.60	68.80	2.5	1.04
7	50.0	13.12	24.00	48.00	21.64	5.19	12.94	13.96	3.35	64.54	2.2	.93
8	53.0	13.91	24.00	45.28	21.44	5.14	12.82	12.91	3.10	60.21	2.0	.85
9	45.0	11.81	20.00	44.44	21.44	4.29	10.70	10.04	2.00	46.82	2.0	.85
10	31.0	8.14	12.00	38.70	21.64	2.59	6.46	9.66	1.16	44.64	1.5	.63
Total or average	381.0	100.00	183.00	48.03	21.91	40.10	100.00	14.17	25.94	64.69	------	1.09
Whole stalk	70.0	------	40.00	57.14	21.84	------	------	15.57	------	71.29	2.4	1.01

[1] Of total.

TABLE 4.—*Yield and composition of juice from various internodes of Hodo variety of sorgo at different stages of maturity*

MILK STAGE

Internode no.	Weight		Juice expressed		Dissolved solids in expressed juice, by Brix			Sucrose in expressed juice		Apparent purity of juice	Acidity expressed in ml of N/10 NaOH	
											Per 10 ml juice	Per gram Brix solids in expressed juice
(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)	(12)	(13)
	Lb.	*Pct.*	*Lb.*	*Pct.*	*Pct.*	*Lb.*	*Pct.*[1]	*Pct.*	*Lb.*		*Cc*	*Cc*
1	17.5	2.56	7.75	44.28	13.54	1.05	2.39	2.23	0.17	16.47	4.0	2.80
2	23.0	3.36	11.00	47.82	15.14	1.66	3.76	5.40	.59	35.67	2.9	1.80
3	32.0	4.68	15.25	47.65	16.04	2.45	5.56	7.44	1.13	46.38	2.1	1.23
4	36.5	5.33	18.25	50.00	16.04	2.93	6.64	7.27	1.33	45.32	1.3	1.21
5	43.0	6.28	20.00	46.51	15.57	3.11	7.05	6.77	1.35	43.48	1.1	.66
6	50.5	7.38	25.00	49.50	14.87	3.72	8.44	6.09	1.52	40.95	1.0	.63
7	62.5	9.13	30.00	48.00	14.17	4.25	9.64	5.27	1.58	37.19	.9	.60
8	71.0	10.37	34.50	48.59	13.97	4.82	10.93	4.59	1.58	32.85	.7	.47
9	70.5	10.30	32.50	46.10	13.52	4.39	9.96	3.70	1.20	27.36	.7	.49
10	74.75	10.92	31.75	41.91	13.22	4.19	9.50	3.12	.99	23.60	.7	.50
11	82.50	12.05	34.50	41.81	12.92	4.45	10.09	3.07	1.06	23.76	.7	.51
12	65.75	9.61	30.00	45.62	12.92	3.87	8.78	2.91	.87	22.52	.7	.51
13	34.50	5.04	16.25	47.10	12.97	2.11	4.79	1.98	.32	15.26	.7	.51
14	20.50	2.99	8.50	41.46	12.87	1.09	2.47	1.74	.15	13.52	.7	.51
Total or average	684.5	100.00	315.25	46.05	13.98	44.09	100.00	4.39	13.84	31.39	------	.72
Whole stalk	70	------	38.50	55.00	13.82	------	------	4.73	------	34.22	1.0	.68

DOUGH-TO-RIPE STAGE

1	13.00	2.38	6.0	46.15	19.08	1.14	2.42	11.21	0.67	58.75	3.0	1.46
2	17.50	3.21	10.0	57.14	19.28	1.93	4.09	11.78	1.18	61.09	2.3	1.10
3	21.50	3.94	12.5	58.13	19.28	2.41	5.11	12.02	1.50	62.34	2.0	.96
4	25.00	4.58	14.5	58.00	18.98	2.75	5.83	11.69	1.69	61.59	1.4	.68
5	30.00	5.49	15.5	51.66	18.63	2.88	6.10	10.41	1.61	55.87	1.3	.64
6	35.00	6.41	19.5	55.71	18.23	3.55	7.52	9.85	1.92	54.03	1.1	.56
7	41.00	7.51	22.5	54.87	17.63	3.96	8.39	8.75	1.97	49.63	1.0	.53
8	46.00	8.42	24.5	53.26	17.33	4.24	8.98	8.06	1.97	46.50	1.0	.53
9	53.00	9.71	25.5	48.11	16.62	4.24	8.98	6.59	1.68	39.65	.9	.50
10	60.50	11.08	30.0	49.58	16.32	4.89	10.36	5.97	1.79	36.58	.9	.51
11	61.50	11.26	27.5	44.71	16.18	4.45	9.43	5.48	1.50	33.86	.9	.52
12	62.50	11.45	28.5	45.60	16.18	4.61	9.77	5.38	1.53	33.25	.9	.52
13	47.00	8.61	21.5	45.74	16.60	3.57	7.57	4.55	.98	27.40	.9	.50
14	32.50	5.95	15.5	47.69	16.60	2.57	5.45	4.49	.69	27.04	.9	.50
Total or average	546.00	100.00	273.50	50.09	17.25	47.19	100.00	7.56	20.68	43.82	------	.60
Whole stalk	75.00	------	44.0	58.66	17.10	------	------	9.69	------	56.66	1.5	.82

DEAD-RIPE STAGE

1	12.0	1.89	6.00	50.00	18.40	1.10	1.92	12.01	0.72	65.27	3.2	1.64
2	19.0	2.99	10.00	52.63	18.45	1.84	3.22	12.39	1.23	67.15	2.4	1.21
3	24.0	3.77	13.00	54.16	18.50	2.40	4.20	12.44	1.61	67.24	2.0	1.00
4	27.5	4.33	14.75	53.63	18.50	2.73	4.77	12.44	1.83	67.24	1.7	.85
5	31.5	4.96	16.50	52.38	18.44	3.04	5.32	12.18	2.01	66.05	1.1	.55
6	31.5	4.96	18.00	57.14	18.04	3.24	5.67	11.83	2.13	65.57	1.0	.51
7	46.0	7.24	23.00	50.00	18.04	4.15	7.26	10.96	2.52	60.75	.8	.41
8	53.0	8.34	27.00	50.94	17.27	4.66	8.15	10.24	2.76	59.29	.6	.33
9	59.0	9.28	30.50	51.69	16.77	5.11	8.94	9.13	2.78	54.44	.6	.33
10	67.5	10.62	34.50	51.11	16.65	5.74	10.04	8.78	3.03	52.73	.7	.39
11	77.0	12.12	40.50	52.59	16.60	6.72	11.75	8.67	3.51	52.22	.7	.39
12	74.0	11.64	39.00	52.70	17.00	6.63	11.60	9.16	3.57	53.88	.7	.38
13	67.0	10.54	34.50	51.49	17.21	5.94	10.39	9.72	3.35	56.47	.7	.38
14	46.5	7.32	22.50	48.38	17.21	3.87	6.77	9.69	2.18	56.30	.8	.43
Total or average	635.5	100.00	329.75	51.89	17.33	57.17	100.00	10.07	33.23	58.10	------	.49
Whole stalk	75.0	------	45.00	60.00	16.55	------	------	9.91	------	59.89	.9	.51

Column 5 gives the ratio, expressed as percentage, of the weight of juice expressed from each batch of internodes to the weight of stalks ground in each batch. The footing of this column is the ratio, expressed as percentage, of the total of column 4 to the total of column 2.

Column 6 lists the percentage of dissolved solids in the juice expressed from each batch of internodes. The footing of this column is the average dissolved solids determined by dividing the total of column 7 by the total of column 4.

Column 7 lists the weight, in pounds, of dissolved solids expressed in the juice from each batch of internodes. This is the product of the degrees Brix in column 6 and the corresponding weight of juice in column 4.

Column 8 lists the percentage of the dissolved solids contributed by each batch of internodes to the total dissolved solids expressed from the stalk. This is arithmetically the ratio, expressed in percentage, of the respective components of column 7 to the total of column 7.

Column 9 lists the percentage of sucrose in the juice expressed from each batch of internodes. The footing of this column is the average obtained by dividing the total of column 10 by the total of column 4.

Column 10 lists the pounds of sucrose in the juice expressed from each batch of internodes. The figures are the product of the percentage of sucrose in column 9 and the corresponding weight of juice in column 4.

Column 11 lists the coefficient of apparent purity,[9] a ratio used in commercial sugar manufacture to estimate sucrose available for recovery by crystallization. The values are obtained by dividing the percentage of sucrose in the juice by the degrees Brix and then multiplying by 100. The footing of this column is the average obtained by dividing the footing of column 9 by the footing of column 6 and multiplying by 100.

Column 12 lists the titratable acidity of the juices. This is expressed in the milliliters of 0.1 normal alkali required to neutralize 10 ml of the juice.

Column 13 lists the acidity in milliliters of 0.1 normal alkali calculated on the basis of 1 g of Brix solids. The figures are obtained by multiplying the volume of juice titrated (10 ml) by the specific gravity [10] at the observed degrees Brix, multiplying the product by the observed degrees Brix, and dividing the result into the titer. The footing of this column is the weighted average obtained by multiplying the figures of column 13 by the corresponding figures in column 7, totaling, and dividing by the total of column 7 expressed in like units.

DISCUSSION

Column 3 of table 3 shows that in the short-stalk, early-maturing variety Iceberg, very nearly three-fourths of the weight of the stalk occurred in internodes 4 to 9, inclusive. Internodes 1, 2, 3, and 10 constituted only about one-fourth of the weight of the stalk. With maturity, there was practically no shift in distribution of weight among the internodes.

In the medium-length, intermediate-maturing varieties Gooseneck and Honey (tables 1 and 2) the bulk of the weight of the stalk, about

[9] SPENCER, G. L., and MEADE, G. P. See pp. 357–358 of reference cited in footnote 8.
[10] SPENCER, G. L., and MEADE, G. P. See pp. 459–468 of reference cited in footnote 8.

two-thirds, occurred in internodes 5 to 10, inclusive, at all three stages of maturity.

In the large-barrel, long-stalk, late-maturing variety Hodo (table 4) about three-fifths of the total weight of the stalk was in internodes 7 to 12, inclusive. There was practically no shift in weight with maturity.

Extraction of juice (column 5) was highest in the upper half of the stalk in all the varieties and at the three stages of maturity. The total juice extracted from the internodes was less than that obtained from the whole stalk. That this was partly due to evaporation of moisture from the relatively short internodes before grinding is shown by the higher average Brix of the juice from the internodes as compared with the Brix of the juice from the whole stalk.

The percentage of dissolved solids (degrees Brix, column 6) increased progressively with maturity in all the varieties with the exception of the dead-ripe stage of the Hodo variety and the upper internodes of the dead-ripe stage of the Honey variety. The Honey variety has a sprangle top, and as soon as the seed head matures and opens, the seeds fall to the ground, branching occurs, and the upper portion of the stalk reverts to the immature stage. The failure of the Hodo variety to show the usual increase in dissolved solids from the dough-to-ripe to dead-ripe stage of maturity was due to intervening rain. This was demonstrated by the fact that although there was little change in the average percentage of dissolved solids of the juice in either the whole stalk or the internode, there was an increase in the percentage of juice expressed.

In the main, the percentage of dissolved solids in the juice increased progressively from the first internode to a maximum in the third or fourth, and decreased progressively to the last internode, except where normal growth had been interfered with by branching or rain.

The percentage of solids in the juice from each internode (based on the total expressed solids, column 8) is a measure of the amount of sirup contributed by the juice of each internode to the total sirup obtained from the stalk. This makes it possible to estimate the loss of sirup incurred by discarding a portion of the stalk.

With the exception of the dead-ripe stage of the Honey variety, the percentage of sucrose determined (column 9) and its ratio to dissolved solids, or coefficient of apparent purity (column 11), increased with maturity. Both reached a maximum in about the same internode as the dissolved solids, but in the lower internodes they decreased much more rapidly than the dissolved solids.

Titratable acidity per gram of dissolved solids in the juice (column 13) is a measure of the acidity contributed by the juice to the sirup manufactured from it. One variety, Hodo (table 4), decreased in average acidity with maturity. The Honey variety (table 2) remained very nearly constant, whereas the Gooseneck and Iceberg varieties (tables 1 and 3) increased with maturity. These variations seem to be varietal characteristics.

The titratable acidity per gram of solids for all varieties and stages of maturity studied was highest in the first internode and decreased rapidly in the internodes constituting the upper third of the stalk. Throughout the remainder of the stalk it remained nearly constant at a value generally less than the weighted whole-stalk average and control.

SUMMARY AND CONCLUSIONS

In four varieties of sorgo at different stages of maturity, two-thirds to three-fourths of the weight of the stalk was derived from internodes in the midportion of the stalk.

With the exception of the sprangle-top variety, Honey, which drops its seed and branches after ripening, with partial reversion in the upper internodes to the immature stage of maturity, and the Hodo variety, the juice of which was diluted by rain between the dough-to-ripe and dead-ripe stages of maturity, the following general trends were noted for the four varieties at different stages of maturity:

The percentage of dissolved solids in the juice increases with maturity, and in the main progressively increases from the first to a maximum in the third or fourth internode, with a small progressive decrease in the lower internodes. There is a close correlation between the percentage of dissolved solids and the percentage of weight contributed by each internode.

The percentage of sucrose in the juice increases from the first to a maximum in the third or fourth internode, and progressively and markedly decreases in the lower internodes. With maturity, the total percentage of sucrose in the expressed juice increases, and in the same relative proportion in the different internodes.

The coefficient of apparent purity of the juice parallels closely the percentage of sucrose determined by polarization. It increases totally with maturity.

If sorgo juices are to be used for the crystallization of sucrose, previously recommended topping practices are incorrect. The reverse procedure should be used, that is, the bottom internodes should be discarded, as they have relatively a much lower coefficient of purity. In some cases several internodes at the bottom of the stalk are much below the practical crystallization limits for sucrose.

In the manufacture of sirup from the sorgo stalk, however, topping, or discarding the three or four upper internodes, reduces the tendency of the sirup to crystallize sucrose and also produces sirup with a minimum of acidity or sharp "tang."

FOOT ROT OF CHINA-ASTER, ANNUAL STOCK, AND TRANSVAAL DAISY CAUSED BY PHYTOPHTHORA CRYPTOGEA [1]

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INTRODUCTION

In August 1934, a destructive foot rot of China-aster (*Callistephus chinensis* Nees) was observed in Golden Gate Park, San Francisco, Calif. Most of the plants which had reached the flowering stage were either dead or dying. The disease, caused by *Phytophthora cryptogea* Pethybr. and Laff., is of general occurrence on the northern end of the San Francisco peninsula. More recently, annual stock or gilliflower (*Matthiola incana* R. Br. var. *annua* Voss), also cultivated extensively in this section, and Transvaal daisy (*Gerbera jamesonii* Hook. var. *transvaalensis* Hort.), grown at Hayward and Capitola, Calif., were found to be similarly affected. The studies reported in this paper deal principally with the disease on China-aster.

REVIEW OF LITERATURE

A disease of China-aster, known as blackleg or foot rot, was reported from England in 1915 by Robinson (*20*),[3] who attributed the cause to an unidentified species of *Phytophthora*.

In 1919, Pethybridge and Lafferty (*16*) described a foot-rot disease of young tomato plants, prevalent in a nursery near Dublin, Ireland. Diseased China-asters with comparable symptoms were found in the same locality. A new species of *Phytophthora*, described as *P. cryptogea*, was cultured from diseased tomato and China-aster plants. The pathogenicity of the two isolates was established by wound inoculations into their respective hosts, while cross inoculations were likewise successful.

Brittlebank and Fish (*6*) reported infection of mature China-aster plants by *Phytophthora cryptogea* in 1927.

The disease was serious on China-asters in England in 1925 and 1926, according to Pethybridge (*15*).

Bewley (*4*) reported the disease on China-asters in England in 1927, and damping-off in 1930.

Pethybridge, Moore, and Smith (*17*) recorded the frequent occurrence of a foot rot of China-aster in 1929 and 1931 in England, and Pethybridge [4] reported its presence in 1933 and 1934.

The first record of the disease on mature China-aster plants in the United States was provided by Tompkins, Tucker, and Clarke (*22*).

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[3] Reference is made by number (italic) to Literature Cited, p. 572.

[4] Letter from George H. Pethybridge dated Aug. 13, 1935.

According to Martin,[5] blackleg of China-asters was found in the District of Columbia in 1926 by Charles Drechsler, who isolated an unidentified species of *Phytophthora* from diseased specimens.

Pethybridge (*15*) and Ware (*23*) have briefly recorded the occurrence of the disease on annual stock or gilliflower (*Matthiola incana* var. *annua*).

Infection of Transvaal daisy (*Gerbera jamesonii* var. *transvaalensis*) has not previously been reported in the literature.

According to published reports, the natural host range of *Phytophthora cryptogea* includes, in addition to China-aster and annual stock, plants of 19 genera in 13 families: Araceae: calla (*Zantedeschia aethiopica* Spreng.) (*11, 17*); Liliaceae: tulip (*Tulipa* sp.) (*1, 10*); Iridaceae: gladiolus (*Gladiolus* sp.) (*1*); Papaveraceae: Iceland poppy (*Papaver nudicaule* L.) (*6*); Cruciferae: cauliflower (*Brassica oleracea* L. var. *botrytis* L.) (*17*), wallflower (*Cheiranthus cheiri* L.) (*6, 16*); Leguminosae: white lupine (*Lupinus albus* L.) (*6*); Umbelliferae: celery seedlings (*Apium graveolens* L.) (*9*); Ericaceae: *Rhododendron carolinianum* Rehd., *R. catawbiense* Michx., *R. maximum* L. (*25*), and *Pieris floribunda* (Pursh) Benth. and Hook.[6]; Primulaceae: primrose (*Primula* sp.) (*6*); Solanaceae: tomato (*Lycopersicum esculentum* Mill. var. *vulgare* Bailey (*2, 6, 8, 16, 17, 21, 24*) and petunia seedlings (*Petunia hybrida* Hort.) (*16*); Scrophulariaceae: snapdragon (*Antirrhinum majus* L.) (*6*); Cucurbitaceae: cucumber (*Cucumis sativus* L.) (*5*); and Compositae: zinnia (*Zinnia elegans* Jacq.) (*12*), dahlia (*Dahlia* sp.) (*17*), African marigold (*Tagetes erecta* L.) (*18*), and cineraria (*Senecio cruentus* DC.) (*3, 6, 16*).[7]

FACTORS FAVORING INFECTION

The principal environmental factors which favor inception and spread of the disease are excessive irrigation, inadequate drainage, and cool weather. The disease is not confined to low, flat areas but may occur on sloping ground if the soil is kept extremely wet. Complete failure of the crop may be expected when soils become waterlogged.

Observations have shown that when plants are grown on well-drained sites and supplied with only enough moisture to satisfy growth requirements, the incidence of disease is greatly reduced.

SYMPTOMS OF THE DISEASE

ON CHINA-ASTER

Damping-off of China-asters, due to *Phytophthora cryptogea*, has not been observed under natural conditions in California, although it has been reported from England (*4, 20*).

Generally the disease does not occur until some weeks after the greenhouse-grown seedlings have been transplanted to beds out of

[5] MARTIN, G. H. DISEASES OF ORNAMENTALS. CALLISTEPHUS CHINENSIS, CHINA-ASTER. U. S. Bur. Plant Indus., Plant Disease Reptr. Sup. 55: 352-363. 1927. [Mimeographed.]

[6] HUMPHREY, H. B., and WOOD, J. I. DISEASES OF PLANTS IN THE UNITED STATES IN 1933. U. S. Bur. Plant Indus., Plant Disease Reptr. Sup. 86, 107 pp. 1934. [Mimeographed.]

[7] After this paper was accepted for publication, attention of the writers was called to a recent report on the natural infection of Marguerite (*Chrysanthemum frutescens* L.) by *Phytophthora cryptogea*. [OYLER, E. A DISEASE OF THE MARGUERITE. Nursery and Market Gard. Indus. Devlpmt. Soc., Chestunt, Expt. and Research Sta. Ann. Rept. (1936) 22:59. 1937.] Accordingly, this host should be added to the list given above.

doors. Although a few plants die early in the season, the heaviest loss occurs immediately preceding and during the flowering period (fig. 1). The disease appears suddenly, and infected plants usually die promptly.

Above ground, the chief diagnostic symptoms of the disease consist of rapid, permanent wilting of the leaves; a blackish-brown discoloration of the lower part of the stem; and lodging of the plant on the ground, occasioned by rotting, shriveling, and collapse of the stem at or near the soil line.

The fungus enters the plant through the tap or lateral roots, ultimately spreading throughout the entire root system and upward into



FIGURE 1.—Phytophthora foot rot of China-asters in Golden Gate Park, San Francisco, August 27, 1935. Infection causes rapid wilting, collapse, and sudden death of the plants.

the lower part of the stem (fig.2). The invaded roots and stems show a soft, water-soaked, blackish-brown, odorless type of decay which at first is localized in the cortical parenchyma but later involves all tissues. Sometimes infected plants develop adventitious roots at the upper edge of the diseased part of the root. Diseased plants are easily pulled from the soil, but invariably the cortex of the taproot and lateral roots sloughs off and remains behind.

ON ANNUAL STOCK

The fungus may attack annual stock plants in all stages of growth, but heaviest infection occurs prior to or during the flowering period. The symptoms consist of sudden wilting and drooping of all but the youngest tuft of leaves, infection and rotting of the roots and lower part of the stem, with ultimate breakage of the stem at or near the soil level. The invaded tissues are blackish brown in color, water-soaked, soft, and odorless. Infected plants are easily pulled from the soil.

ON TRANSVAAL DAISY

On Transvaal daisy, symptoms consist of sudden wilting of the leaves accompanied by a conspicuous color change from the normal green to the violet carmine of Ridgway (*19*). Plants of all ages are

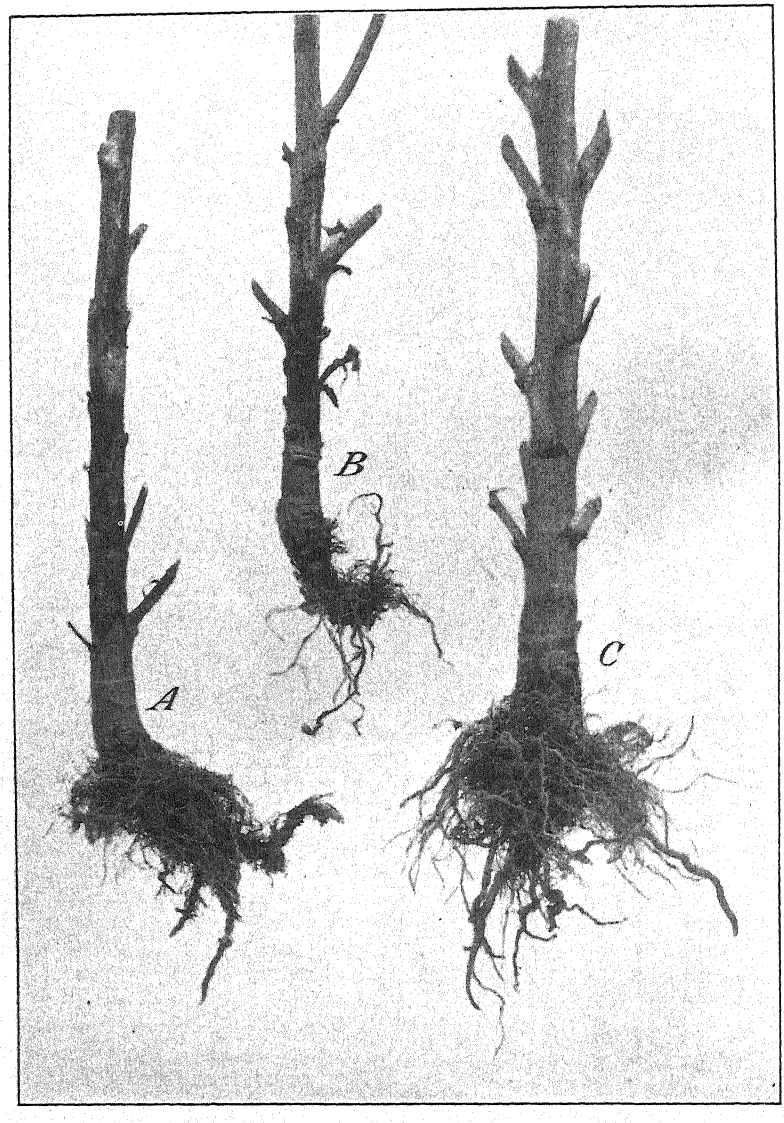


FIGURE 2.—Phytophthora foot rot of China-aster (natural infection): *A* and *B*, Decay of the root system and typical lesions on the lower part of the stems; *C*, healthy root system and stem

susceptible, but the principal damage occurs during the flowering period. After invading the crown tissues at or just below the soil level, the fungus travels downward into the roots. Diseased crown and root tissues are soft, water-soaked, blackish brown in color, and

odorless. When infected plants are dug, the cortex of the roots usually sloughs off, exposing the discolored central cylinder or stele. If an attempt is made to pull the plant before digging, breakage occurs at the crown and most of the roots remain in the soil.

THE CAUSAL FUNGUS, PHYTOPHTHORA CRYPTOGEA

Phytophthora cryptogea was readily isolated from the roots and stems of recently infected China-aster, annual stock, and Transvaal daisy plants by placing small tissue fragments from the internal, advancing margin of decay on malt-extract agar (*13*). After 48 hours, pure cultures were established on agar slants by transferring hyphal tips from Petri-dish colonies. The fungus was cultured at intervals from approximately 100 diseased China-aster and 50 diseased annual stock plants collected in different localities on the San Francisco peninsula. It was also isolated from numerous diseased Transvaal daisy plants. From a group of diseased Transvaal daisy plants collected at Burlingame, Calif., pure cultures of *P. drechsleri* Tucker were consistently obtained.

Freehand cross and longitudinal sections of naturally and artificially infected roots and stems of China-aster, annual stock, and Transvaal daisy were stained with fast green and Magdala red. The fungus is intercellular. Pethybridge and Lafferty (*16*) found the mycelium to be intercellular and intracellular in the parenchymatous tissues of tomato plants. The hyphae, upon invading healthy tissues, were confined to the intercellular spaces, but in very young seedlings and in older diseased plants were observed in the wood vessels.

Isolates of *Phytophthora cryptogea* from China-aster, annual stock and Transvaal daisy were indistinguishable in culture. On potato-dextrose agar there was scant aerial growth of white mycelium with the development of occasional clusters of vesicular swellings in the mycelium; on Difco corn-meal agar the mycelial growth was almost entirely submerged; on oatmeal agar the development of aerial mycelium was fairly profuse but no spores appeared. Sporangia and oogonia failed to develop on any of the solid culture media used.

The vesicles that appeared in potato-dextrose agar cultures were usually less than 27 microns in diameter. They were spherical to irregular in shape, thin-walled, with hyaline contents that resembled those of the hyphae, from which they were not separated by walls as are the chlamydospores produced by *Phytophthora parasitica* and other species. The vesicles of *P. cryptogea* differed from those of *P. cinnamomi* in their smaller size, comparative rarity in culture, and in their smaller degree of differentiation from the hyphae.

Sporangia developed fairly abundantly on hyphae transferred from week-old cultures on oatmeal agar or in pea broth to Petri's solution, distilled water, or nonsterile soil leachate, as suggested by Mehrlich (*14*); in the latter the sporangia developed very promptly, appearing after 48 hours. They were obpyriform to ovate or elongated, the latter often slightly constricted near the middle, non-papillate, with a small distinct refringent thickening at the apical end, 22.8 to 44.8 by 11.6 to 21.2 microns, averaging 32.2 by 17.2 microns. Sporangia produced after 6 days in Petri's solution were similar in shape and size to those produced in the nonsterile soil leachate. The

sporangia germinated by the growth of a single slender hypha from the refringent apical region, or by the development of zoospores which were fully differentiated within the sporangium and escaped in the manner typical of the genus. The sporangiophores resumed growth through evacuated sporangia in the fashion usual in *Phytophthora cryptogea* and certain other species with nonpapillate sporangia.

Oogonia, oospores, and antheridia were not observed in the cultures examined, and late and scanty development of sexual spores in culture is probably characteristic of the species.

Nine isolates were grown on Difco corn-meal agar plates at various temperatures. After 96 hours the average diameters of the mycelial growths were as follows: At 3° to 4.5° C., 0; at 11°, 15 mm; at 15°, 35 mm; at 20°, 50 mm; at 25°, 59 mm; at 30°, 48 mm; at 35°, 0. At 35° the inocula usually failed to survive the exposure of 96 hours.

The California isolates of *Phytophthora cryptogea* were compared in parallel cultures with an isolate of the species sent to the Centraalbureau voor Schimmelcultures by Pethybridge. Their similarity in cultural characters, morphology, and temperature relations seem to justify the identification of the California isolates with the aster parasite reported from Europe and Australia, even in the absence of observations on the sexual stages.

PATHOGENICITY TESTS

Inoculum was prepared by growing the different isolates of the fungus on sterilized, moistened cracked wheat. It was added in uniform quantity to autoclaved soil in 6-inch pots, each containing a young plant (6 to 10 leaves), in a manner designed to avoid wounding the roots. Sterilized wheat was used for the noninoculated controls. The plants, grown in a greenhouse at temperatures ranging from 18° to 22° C., were watered heavily each day to provide favorable conditions for infection.

ON CHINA-ASTER

The pathogenicity of the China-aster isolate was proved on the variety Late Branching Mary Semple. On December 28, 1934, 25 plants were inoculated. By January 9, 1935, all plants were infected, the incubation period ranging from 5 to 12 days. Symptoms shown by diseased plants were identical with those resulting from natural infection. Typical wilting of the leaves (fig. 3) and collapse of the stem with lodging of the plant occurred. When diseased plants were pulled from the soil, the soft, watery, blackish-brown cortical tissues were readily sloughed off from the central cylinder of the taproot. Most of the lateral roots were completely rotted. The five noninoculated controls continued healthy. All infected roots yielded the fungus when plated.

On January 25, 25 China-aster plants of the same variety were inoculated with the reisolated fungus. All plants were infected by February 5, the incubation period ranging from 7 to 11 days. The five noninoculated controls were healthy.

Damping-off of China-aster seedlings (varieties Apricot and Fordhook Favorite) occurred when seeds were planted in previously infested soil or in autoclaved soil to which the fungus was later added.

ON ANNUAL STOCK

Healthy annual stock plants (variety Fiery Blood Red) were readily infected in 24 days by artificial inoculation with the isolate from this host (fig. 4). The fungus was reisolated and its pathogenicity again demonstrated by inoculation. All control plants remained healthy.

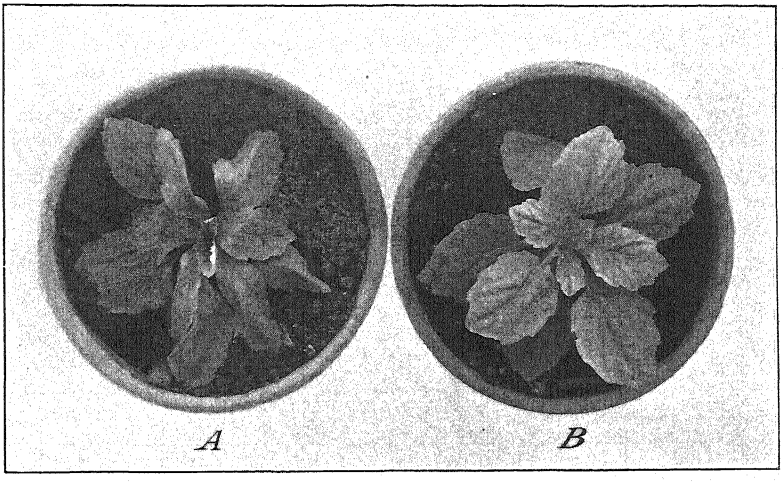


FIGURE 3.—Phytophthora foot rot of China-aster: *A*, Artificial infection of the variety Late Branching Mary Semple after 7 days, showing rapid wilting; *B*, healthy control.

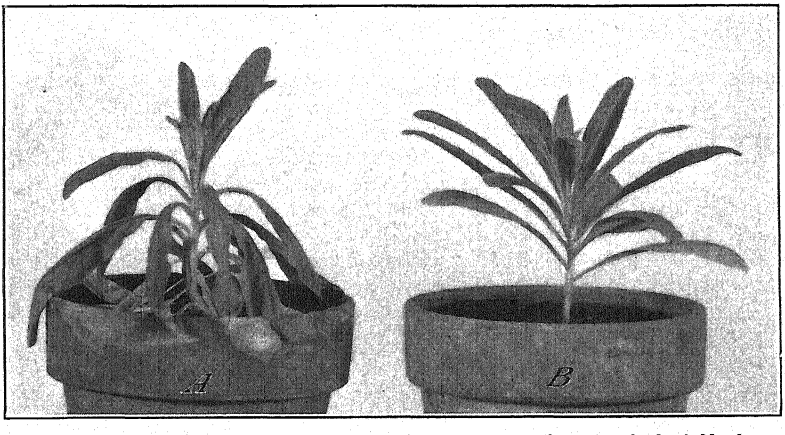


FIGURE 4.—Phytophthora foot rot of annual stock: *A*, Rapid wilting of the outer whorls of older leaves 20 days after inoculation with the isolate from this host, a tuft of young inner leaves remaining turgid; *B*, healthy control. Variety Fiery Blood Red.

ON TRANSVAAL DAISY

Two isolates of *Phytophthora cryptogea* from Transvaal daisy grown in widely separated localities proved pathogenic when inoculated into young, healthy Transvaal daisy plants. Typical symptoms of the disease were produced in 20 to 69 days. The pathogenicity of the two reisolates was again demonstrated by inoculation.

A culture of *Phytophthora drechsleri*, previously referred to, also caused infection of healthy Transvaal daisy plants after inoculation. Infected plants showed symptoms which were identical with those resulting from invasion by *P. cryptogea*, and the incubation period was approximately of the same duration. The desirability of laboratory cultural examinations of diseased specimens is apparent.

It is concluded that the several isolates of the fungus are highly pathogenic to the hosts from which they originated. That direct penetration of unwounded healthy roots and stems occurred under conditions of these tests is indicated.

SUSCEPTIBILITY OF CHINA-ASTER VARIETIES TO INFECTION

Numerous commercial varieties of China-aster were tested for susceptibility to infection by *Phytophthora cryptogea*. For convenience, these varieties were segregated into arbitrary classes:[8] Crego, 36; Dwarf Bouquet, 1; Early Beauty, 24; Giants of California, 14; King, 14; Late Beauty, 4; Late Branching, 39; Peony Flowered, 11; Pompon, 17; Queen of the Market, 20; Royal, 9; Single or Sinensis, 11; Sunshine, 4; Victoria, 10; and unclassified, 13. Of the 227 varieties, 75 were listed as resistant to fusarium wilt by the seedmen from whom they were obtained. Wilt-resistant varieties were not available in the Dwarf Bouquet, Giants of California, Late Beauty, Pompon, and Sunshine classes. In addition, one foreign variety belonging to the King class and of no commercial value because of its dwarf habit, was tested.

Approximately 20 plants of each variety were inoculated according to the method previously described. This involved in the aggregate about 5,000 plants. All varieties proved to be highly susceptible, with no survival of individuals in any particular variety. Under greenhouse conditions, the fusarium wilt-resistant varieties were as readily attacked by the fungus as those listed as wilt susceptible. The incubation period for most varieties ranged from about 10 to 45 days. When considered collectively, the minimum incubation period for all tested varieties was 6 days and the maximum 93 days. Although some infected plants reached the flowering stage before they died, most of them succumbed prior to flower formation.

In the autumn of 1935, seed was obtained from a few apparently resistant China-aster plants grown in Golden Gate Park. Progeny of all selections proved susceptible upon inoculation.

EXPERIMENTAL HOST RANGE

Artificial infection of certain plants by *Phytophthora cryptogea* has been recorded by several investigators, a brief resume of which is given. Pethybridge and Lafferty (*16*) reported infection with an isolate of this fungus from tomato, after wounding, of beech seedlings (*Fagus sylvatica* L.), mangels (*Beta vulgaris* L.), swedes (*B. campestris* L. var. *napobrassica* DC.), white turnips (*B. rapa* L.), apples (*Malus sylvestris* Mill.), *Gilia tricolor* Benth., potato tubers (*Solanum tuberosum* L.), and green and ripe tomato fruits (*Lycopersicum esculentum* var. *vulgare*). Later, Cairns and Muskett (*7*) corroborated in part the earlier work of Pethybridge and Lafferty (*16*) by artificially producing pink rot of potato tubers (*S. tuberosum*).

[8] This classification was kindly suggested by Harry B. Joy of Salinas, Calif.

The China-aster isolate of *Phytophthora cryptogea* has a comparatively limited experimental host range. Young plants (6 to 10 leaves) of wallflower (*Cheiranthus cheiri*), annual stock (*Matthiola incana* var. *annua*), Transvaal daisy (*Gerbera jamesonii* var. *transvaalensis*), Michaelmas daisy (*Aster alpinus* L., *A. oreophilus* Franch., and *A. subcoeruleus* S. Moore var. *diplostephioides* Hort.), and hybrid cineraria (*Senecio cruentus*) were artificially infected. Damping-off of cucumber seedlings (*Cucumis sativus*) occurred when seeds were planted in infested soil.

Fruits of eggplant (*Solanum melongena* L. var. *esculentum* Nees), ripe tomato (*Lycopersicum esculentum* var. *vulgare*) var. Stone, green bell pepper (*Capsicum annuum* L. var. *grossum* Sendt.), pumpkin (*Cucurbita pepo* L. var. *condensa* Bailey) var. Early White Bush Scallop, watermelon (*Citrullus vulgaris* Schrad.), and cucumber (*Cucumis sativus*) were infected without wounding. Lesions on all fruits had a water-soaked appearance except those on eggplant, which were warm sepia in color.

The following species reacted negatively to artificial inoculation: Bulbous plants, including Chinese sacred-lily (*Narcissus tazetta* L. var. *orientalis* Hort.), tulip (*Tulipa gesneriana* L.), hyacinth (*Hyacinthus orientalis* L.), hybrid gladiolus (*Gladiolus* sp.), freesia (*Freesia hybrida* Hort.), and poppy-flowered anemone (*Anemone coronaria* L.); fruits of apple (*Malus sylvestris* Mill.), var. Yellow Newtown, green tomato (*Lycopersicon esculentum* var. *vulgare*) var. Stone, squash (*Cucurbita maxima* Duchesne) var. Banana, pumpkin (*Cucurbita pepo* var. *condensa*) var. Pie, Yellow Crookneck, and Zucchine, Persian melon (*Cucumis melo* L. var. *reticulatus* Naud.), and Honey Dew melon (*C. melo* var. *inodorus* Naud.); roots of garden beet (*Beta vulgaris*), rutabaga (*B. campestris* var. *napobrassica*), turnip (*B. rapa*) var. Purple Top White Globe, carrot (*Daucus carota* L.), and parsnip (*Pastinaca sativa* L.); tubers of potato (*Solanum tuberosum*) var. Russet Burbank; and young plants of Iceland poppy (*Papaver nudicaule*), wallflower (*Cheiranthus cheiri*), pansy (*Viola tricolor* L.), celery (*Apium graveolens*) var. Golden Self Blanching, common pimpernel or poor-man's-weatherglass (*Anagallis arvensis* L.), *Gilia tricolor*, tomato (*Lycopersicum esculentum* var. *vulgare*) var. Early Santa Clara Canner, green bell pepper (*Capsicum annuum* var. *grossum*), tobacco (*Nicotiana tabacum* L. var. Turkish), petunia (*Petunia hybrida*), snapdragon (*Antirrhinum majus*), nemesia (*Nemesia strumosa* Benth. var. *suttonii* Hort.), sunflower (*Helianthus annuus* L.), zinnia (*Zinnia elegans*) var. Double Lilliput Purple, English daisy (*Bellis perennis* L.), Michaelmas daisy (*Aster farreri* W. M. Sm., *A. horizontalis* hort. hybr. *grandiflorus*, *A. pyramidalis*, *A. subcoeruleus* S. Moore, and *A. yunnanensis* Franch.), French marigold (*Tagetes patula* L.), and African marigold (*T. erecta* L.).

Seeds of several species were planted in infested soil but no damping-off occurred on seedlings of cauliflower (*Brassica oleracea* var. *botrytis*) var. February, turnip (*B. rapa*) var. Purple Top White Globe, wallflower (*Cheiranthus cheiri*, celery (*Apium graveolens*), parsnip (*Pastinaca sativa*), *Gilia tricolor*, tomato (*Lycopersicum esculentum* var. *vulgare*), petunia (*Petunia hybrida*), snapdragon (*Antirrhinum majus*), zinnia (*Zinnia elegans*), dahlia (*Dahlia* sp.), and hybrid cineraria (*Senecio cruentus*).

Two varieties of China-aster (Giant Crego Azure Blue, wilt resistant, and Queen of the Market Lavender, wilt resistant) were readily infected by the isolate of *Phytophthora cryptogea* from annual stock after 17 days. However, the type culture from Pethybridge (host not specified), an isolate from African marigold (*18*), and the two isolates from Transvaal daisy did not cause infection after 41 days.

Plants of annual stock (variety Fiery Blood Red) were infected with the two isolates from Transvaal daisy after 23 days. The type culture yielded negative results.

Successful inoculations were made to Transvaal daisy plants with the isolate from annual stock, after 29 days, and with the isolate from marigold, after 22 days. The type culture did not cause infection.

SUMMARY

A foot rot of China-aster, annual stock, and Transvaal daisy, caused by *Phytophthora cryptogea* Pethybr. and Laff., is prevalent on the San Francisco peninsula and in other localities.

The principal environmental factors favoring the disease are excessive moisture, poor soil drainage, and cool weather.

Infected plants wilt very suddenly. The roots and the lower part of the stems of China-aster and annual stock and the roots and crowns of Transvaal daisy are involved in a soft, wet decay. Ultimately breakage of the stems or crowns at or near the soil level is followed by lodging and death of the plants.

The isolates of the fungus from China-aster and other hosts are described.

In the greenhouse, infection was obtained by adding the fungus to the wet, autoclaved soil of potted plants. The incubation period averaged 10 days for China-aster, 24 days for annual stock, and 45 days for Transvaal daisy.

The China-aster isolate was pathogenic to cucumber seedlings and young plants of wallflower, annual stock, Transvaal daisy, Michaelmas daisy, and hybrid cineraria, as well as to unwounded fruits of eggplant, ripe tomato, green bell pepper, pumpkin, watermelon, and cucumber.

The annual stock isolate caused infection of China-aster and Transvaal daisy.

When inoculated into annual stock, the Transvaal daisy isolates proved pathogenic, but no infection of China-aster was obtained.

An isolate of *Phytophthora drechsleri* from Transvaal daisy readily infected that host. Symptoms produced were indistinguishable from those induced by *P. cryptogea*.

No resistance to the disease was found in any of the commercial varieties of China-asters tested, including strains resistant to fusarium wilt.

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A FORMULA FOR REDUCING THE COMPUTATIONS NECESSARY TO FIND THE VARIANCE OF A SET OF AVERAGES[1]

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The yield of a three-row plot is often considered to be the average of the yields of the rows. The averages of these yields are used to obtain the mean and variance of the yields of the entire number of plots used in the experiment. Since the yield of each plot is an average of three-row yields, fractions are usually involved; also, deviations of the plot yields from the general mean of the group involve more fractions which increase the work of computation.

The weight of a steer is often considered to be the average of three measurements of his weight taken at different times during the day. Some research workers find the average of the three measurements, the deviations of these averages from the general average, and then the variance of the measurements from the sum of squares of these deviations. This method is used frequently in finding the variance of the weights of steers employed in a project. When this method of calculating the variance is employed, a great amount of computing is usually required in which decimal fractions arise.

The object of this paper is to show how to obtain the mean and variance of the measurements without the labor involved in finding the averages of the three measurements made at the outset and the deviations of these averages from the general mean.

Let x_1, y_1, z_1 represent, respectively, the yields of the three rows in the first plot; let x_2, y_2, z_2 represent the yields of the three rows in the second plot, etc. Yields of the plots are taken as the average of the three-row yields. They are, respectively,

$$W_1=\frac{x_1+y_1+z_1}{3},$$

$$W_2=\frac{x_2+y_2+z_2}{3},$$

$$W_3=\frac{x_3+y_3+z_3}{3},$$

$$\vdots \qquad \vdots$$

$$W_n=\frac{x_n+y_n+z_n}{3}.$$

The general mean, or the mean of all the plot yields, is

$$M=\frac{\Sigma W}{n}=\frac{\Sigma(x+y+z)}{3n}=\frac{\Sigma x+\Sigma y+\Sigma z}{3n},$$

which is the sum of all row yields divided by $3n$, the total number of rows in all plots.

[1] Received for publication Mar. 15, 1937; issued November, 1937. Journal Article no. 294 (n. s.) from the Michigan Agricultural Experiment Station.

It is not necessary to use the actual measurements, for by using an assumed or provisional mean the general mean and variance can be found. The use of a provisional mean enables one to reduce the size of the items and thus to lessen the chances of making errors. Let m be the provisional mean, which is usually some whole number near the center of the range of the measurements; let x_1' be the deviation of the yield of the first row in the first plot from the provisional mean, m; let y_1' be the deviation of the yield of the second row in the first plot from m, and z_1' the deviation of yield of the third row in the first plot from m; etc. The deviations of the original measurements from the provisional mean, m, are small numbers and hence are easier to use in computing.

The general mean, M, can be written in terms of the deviations from the provisional mean. By definition

$$\begin{aligned} &x_1'=x_1-m,\ y_1'=y_1-m,\ x_1'=z_1-m \\ &x_2'=x_2-m,\ y_2'=y_2-m,\ z_2'=z_2-m \\ &\vdots\quad\ \vdots\quad\ \vdots\quad\ \vdots\quad\ \vdots\quad\ \vdots \\ &x_n'=x_n-m,\ y_n'=y_n-m,\ z_n'=z_n-m. \end{aligned}$$

Adding the above gives,

$$\Sigma(x_i'+y_i'+z_i')=\Sigma(x+y+z)-3nm \tag{1}$$

or

$$\Sigma(x+y+z)=\Sigma(x_i'+y_i'+z_i')+3nm$$

Hence

$$M=\frac{\Sigma(x+y+z)}{3n}=\frac{\Sigma(x'+y'+z')}{3n}+m \tag{2}$$

From the definition

$$x_i+y_i+z_i=x_i'+y_i'+z_i'+3m \tag{3}$$

The variance [2] of the yields of the plots, obtained by taking the average of the three measurements, is by definition

$$\sigma^2=\sum_{i=1}^{n}\left(\frac{x_i+y_i+z_i}{3}-\sum_{i=1}^{3n}\left(\frac{x_i+y_i+z_i}{3n}\right)\right)^2 \Big/ (n-1)$$

or

$$\sigma^2=\sum_{i=1}^{n}\left(\frac{n(x_i+y_i+z_i)-\sum_{i=1}^{3n}(x_i+y_i+z_i)}{3n}\right)^2 \Big/ (n-1) \tag{4}$$

Substituting the values of $\Sigma(x_i+y_i+z_i)$ and $x_i+y_i+z_i$ from (2) and (3) respectively into (4), gives

$$\sigma^2=\sum_{i=1}^{n}\left(\frac{n(x_i'+y_i'+z_i')+3nm-\sum_{i=1}^{3n}(x_i'+y_i'+z_i')-3nm}{3n}\right)^2 \Big/ (n-1) \tag{5}$$

$$\therefore\ \sigma^2=\frac{1}{(n-1)}\sum_{i=1}^{n}\left(\frac{n(x_i'+y_i'+z_i')-\sum_{i=1}^{3n}(x_i'+y_i'+z_i')}{3n}\right)^2.$$

[2] This is considered to be the best estimate of the variance of the population from which the sample is taken.

Now, carrying out the summation in the above line, we have the following without the denominators

$$n^2(x_1'+y_1'+z_1')^2-2n\Sigma(x_j'+y_j'+z_j')(x_1'+y_1'+z_1')+(\Sigma(x_j'+y_j'+z_j'))^2$$
$$n^2(x_2'+y_2'+z_2')^2-2n\Sigma(x_j'+y_j'+z_j')(x_2'+y_2'+z_2')+(\Sigma(x_j'+y_j'+z_j'))^2$$
$$\vdots$$
$$n^2(x_n'+y_n'+z_n')^2-2n\Sigma(x_j'+y_j'+z_j')(x_n'+y_n'+z_n')+(\Sigma(x_j'+y_j'+z_j'))$$

Adding, we get

$$n^2\sum_{i=1}^{n}(x_i'+y_i'+z_i')^2-2n\sum_{j=1}^{n}(x_j'+y_j'+z_j')\sum_{j=1}^{n}(x_j'+y_j'+z_j')+n\left(\sum_{j=1}^{n}(x_j'+y_j'+z_j')\right)^2$$

$$\therefore\ \sigma^2=\left\{\frac{n^2\sum_{i=1}^{n}(x_i'+y_i'+z_i')^2-n\left(\sum_{i=1}^{n}(x_j'+y_j'+z_j')\right)^2}{9n^2}\right\}\Big/(n-1)$$

or

$$(6)\qquad \sigma^2=\left\{\frac{n\sum_{i=1}^{n}(x_i'+y_i'+z_i')^2-\left(\sum_{j=1}^{n}(x_j'+y_j'+z_j')\right)^2}{9n}\right\}\Big/(n-1)$$

Formula (6) looks complicated; actually it is very easy to use, as table 1 concerning yields of three-row plots of Minhardi wheat will show. The first three columns in table 1 contain wheat yields for the three rows of 10 plots. The next three columns contain the deviations of the yields from the provisional mean, 250 g. In column 7 are recorded the sum of the items in columns 4, 5, and 6; the last column contains the squares of the numbers in column 7.

TABLE 1.—*Data to illustrate the use of formula (6)*

Yield of Minhardi wheat			$x-250$	$y-250$	$z-250$	$x'+y'+z'$	$(x'+y'+z')^2$
Row 1 x	Row 2 y	Row 3 z	x'	y'	z'		
Grams	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*
247	251	224	−3	1	−26	−28	784
249	223	233	−1	−27	−17	−45	2,025
271	252	221	21	2	−29	−6	36
263	233	255	13	−17	5	1	1
280	215	260	30	−35	10	5	25
255	234	258	5	−16	8	−3	9
256	217	241	6	−33	−9	−36	1,296
262	226	268	12	−24	18	6	36
297	267	284	47	17	34	98	9,604
288	252	269	38	2	19	59	3,481
Total						+51	17,297

From (2) the value of the general mean is $M=250+\frac{51}{30}=251.7$ g.

From formula (6) the variance of this set of data, where the plot yield is considered to be the average of the yields of the three rows in the plot, is

$$\sigma^2=\frac{10(17{,}297)-(51)^2}{90\ (9)}=\frac{170{,}369}{90(9)}=210.3$$

$$\therefore \sigma=\sqrt{210.3}=14.5\text{g}$$

In the foregoing illustration there were no fractions in the calculations until the last step in finding M and the last division in finding σ^2.

A similar formula can be derived from n plots, each with k rows, where the yield of a plot is considered to be the average of the yields of the k rows. The formulas for mean and variance are

$$M=\frac{\sum_{i=1}^{n}(a_i'+b_i'+c_i'+\cdots+k_i')}{k\cdot n}+m \tag{7}$$

and

$$\sigma^2=\frac{n\sum_{i=1}^{n}(a_i'+b_i'+c_i'+\cdots+k_i')^2-\left(\sum_{i=1}^{n}(a_i'+b_i'+c_i'+\cdots+k_i')\right)^2}{k^2\cdot n(n-1)} \tag{8}$$

where a_i is the yield of the first row of the ith plot, and a_i' is the deviation of the yield of the first row in the ith plot from the provisional mean m.

Let us now consider a set of weight measurements made on 13 steers, where the weight of a steer is considered to be the average of five measurements made during the first day of the feeding experiment, as shown in table 2.

TABLE 2.—*Data to illustrate the use of formulas (7) and (8)*

Steer no.	Weights of steers taken at 5 times during first day					$m=310$					(h) $a'+b'+c'+d'+e'$	$(h)^2$
	First a	Second b	Third c	Fourth d	Fifth e	$a-310$ a'	$b-310$ b'	$c-310$ c'	$d-310$ d'	$e-310$ e'		
	Lb.	*Lb.*	*Lb.*	*Lb.*	*Lb.*	*Lb.*	*Lb.*	*Lb.*	*Lb.*	*Lb.*	*Lb.*	*Lb.*
1	300	315	304	308	310	−10	5	−6	−2	0	−13	169
2	285	280	293	297	288	−25	−30	−17	−13	−22	−107	11,449
3	325	322	317	329	320	15	12	7	19	10	63	3,969
4	337	324	330	334	328	27	14	20	+24	18	103	10,609
5	295	307	301	299	306	−15	−3	−9	−11	−4	−42	1,764
6	271	283	280	278	285	−39	−27	−30	−32	−25	−153	23,409
7	305	319	312	318	306	−5	9	2	8	−4	10	100
8	329	322	314	319	324	19	12	4	9	14	58	3,364
9	304	309	317	321	314	−6	−1	7	11	4	15	225
10	325	333	335	340	330	+15	23	25	30	20	113	12,769
11	301	313	297	303	307	−9	3	−13	−7	−3	−29	841
12	279	288	291	284	275	−31	−22	−19	−26	−35	−133	17,689
13	298	294	306	301	290	−12	−16	−4	−9	−20	−61	3,721
Total											−176	90,078

From (7) the mean of the weights, where weight is considered to be the average of the five weights made during the first day, is

$$M=\frac{(-176)}{5\times 13}+310=307.29 \text{ pounds.}$$

From (8) the variance is

$$\sigma^2=\frac{13(90{,}078)-(-176)^2}{25\times13\times12}=292.32$$

and

$$\sigma=17.1 \text{ pounds.}$$

This formula may be employed to shorten computation work when Harris' formula is used for finding the intraclass correlation coefficient. Harris' [3] formula is

$$k\sum_{p=1}^{n'}(\bar{x}_p-\bar{x})^2=ns^2.[1+(k-1)n], \tag{9}$$

where k=the number of items in the same family; n'=the number of families;
$\bar{x}$=the mean of all items; n=the number of degrees of freedom;
s^2=the variance of an entire set of items; $\bar{x}_p$=the mean of the items in the p th family: r=the intraclass correlation.

The summation, $\sum_{p=1}^{n'}(\bar{x}_p-\bar{x})^2$, in formula (9) can be found by formula (6), as the following example shows.

The number of pedicels per inflorescence of nonterminal inflorescences on the second branch of a certain plant were counted for obtaining the correlation between number of pedicels for sister clusters. There were three nonterminal inflorescences on the second branch of these plants. Table 3 gives the data.

TABLE 3.—*Data to illustrate the use of formulas (6) and (9)*

Pedicels per cluster			$m=8$			$x'+y'+z'$	$(x'+y'+z')^2$
First cluster x	Second cluster y	Third cluster z	x'	y'	z'		
Number	*Number*	*Number*					
8	9	8	0	1	0	1	1
10	10	9	2	2	1	5	25
7	8	7	−1	0	−1	−2	4
9	7	8	1	−1	0	0	0
6	7	6	−2	−1	−2	−5	25
8	9	9	0	1	1	2	4
8	8	8	0	0	0	0	0
7	7	6	−1	−1	−2	−4	16
10	9	8	2	1	0	3	9
8	9	9	0	1	1	2	4
7	6	8	−1	−2	0	−3	9
11	8	9	3	0	1	4	16
9	9	9	1	1	1	3	9
8	8	8	0	0	0	0	0
7	6	6	−1	−2	−2	−5	25
9	8	8	1	0	0	1	1
7	7	7	−1	−1	−1	−3	9
11	11	10	3	3	2	−8	64
8	8	7	0	0	−1	−1	1
10	8	10	2	0	2	4	16
Total						−6	238

[3] Harris, J. A. A CONTRIBUTION TO THE PROBLEM OF HOMOTYPOSIS. Biometrika 11: 201–214, illus. 1916.

By using the quantity in brackets in formula (6) the sum, $\Sigma(\bar{x}_p-\bar{x})^2$, is equal to

$$\Sigma(\bar{x}_p-\bar{x})^2=\frac{20(238)-(6)^2}{9\cdot 20}=\frac{4724}{180}.$$

This value used in formula (9) leads to an intraclass correlation coefficient of 0.925.

Formulas (7) and (8) enable one to find the mean and the standard deviation of a set of averages without actually finding the averages or the deviations from the general mean and by employing smaller numbers than the original measurements. These formulas reduce the work of computation.

THE URONIC ACID CONTENT OF THE NITROGEN-FREE EXTRACT OF FEEDING STUFFS [1]

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INTRODUCTION

Abundant data exist on the percentage of nitrogen-free extract in feeds, but in only a few instances is there information concerning the kind and quantity of carbohydrates comprising it. According to their digestibility, Fraps (*6*)[2] arranged the known components of the nitrogen-free extract in the following order: (1) Sugars, (2) starch, (3) pentosans, (4) residue. It is also being recognized that the effects of association of the different components may have a great deal to do with digestibility. Thus, results (*5*, *6*, *7*, *8*, *9*, *13*, *14*) show that the sugars and starches and the pentosans of hays and forages are generally less digestible than the same constituents in starchy concentrates and meal feeds.

The term "residual nitrogen-free extract" is given by Fraps (*10*) to that part of the nitrogen-free extract remaining after the values for sugars, starch, and pentosans have been subtracted. This fraction includes the uronic acids, with the determination of which this paper deals.

Polymers of uronic acids are widespread in the plant world as incrusting materials and as constituents of cell walls, and in the hemicellulose and pectic materials. Link (*15*, *16*) has demonstrated the presence of free glycuronic acid in corn seedlings. It is well known that compounds like benzoic acid and certain terpene derivatives are eliminated from the higher animals as "paired glycuronic acids." Mathews (*17*) suggests that the uronic acid is a transitory stage in the oxidation of glucose in the body; however, it has never been shown that the higher animals are able to utilize the uronic complexes.

METHODS OF EXPERIMENTATION

MATERIALS

Nine feeds representing concentrates, grain byproducts, and roughage were selected to give a variety with respect to physical and chemical characteristics, as is shown in table 1. The peanuts were purchased in the open market; the other materials were available at the Minnesota Agricultural Experiment Station. All were obtained in an air-dried condition. In each case a 300-g sample was coarsely ground and then placed in a ball mill until the particles were reduced to a size passing through a sieve having circular openings of 1 mm. diameter.

[1] Received for publication June 11, 1936; issued November 1937. Contribution no. 1428 of the Minnesota Agricultural Experiment Station.

[2] Reference is made by number (italic) to Literature Cited, p. 585.

TABLE 1.—*Composition of feeding stuffs (moisture free)*

Feed	Crude fiber	Pentosans in crude fiber	Nitrogen-free extract	Uronic acid anhydride	Sugars and starch			
					Reducing sugars	Sucrose	Starch	Total
	Percent	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
Alfalfa hay	30.31	5.03	40.37	10.40	1.76	1.65	1.44	4.85
Corncobs	36.53	4.48	58.87	5.88	1.44	.60	2.03	4.07
Corn bran	16.96	1.16	62.46	6.64	.30	nil	7.34	7.64
Peanut hulls	76.40	14.47	14.70	6.00	.83	.48	.88	2.11
Peanut kernels	2.43	.20	14.95	1.92	(1)	5.39	3.27	8.66
Rye	2.21	.15	81.36	.72	.17	5.43	53.68	59.28
Timothy hay	34.55	5.98	47.19	5.00	3.57	1.39	.74	5.70
Wheat bran	12.11	.99	53.23	3.88	.70	3.91	7.83	12.44
Wheat shorts	6.74	.49	68.96	1.68	1.16	4.34	20.73	26.23

[1] Trace.

ANALYTICAL METHODS

The uronic acid content was determined by the method of Dickson, Otterson, and Link (*4*) except that the liberated carbon dioxide was received in two spiral tubes (Bailey (*3*)) in series, and the excess of barium hydroxide titrated in these containers. Approximately 20 minutes of heating is required to bring the temperature of the reaction mixture to 100° C., during which time any carbon dioxide evolved from carbonates present is removed. At this stage the standardized solution of barium hydroxide is introduced into the receiving train. This is a slight modification of Anderson's method (*1*). Norris and Resch (*18*) have recently verified the statement that the determination of uronic acids by decarboxylation is quantitative. In the case of the rye, wheat shorts, and wheat bran samples, which contain large quantities of starch and sugars, correction was made for the carbon dioxide evolved under the conditions of the determination (0.45 percent of the weight of the carbohydrate). The quantities of protein present do not yield a measurable amount of carbon dioxide under the above conditions.

The determinations of moisture, ash, crude protein, pentosans, sucrose, reducing sugars, starch, and ether extract were made by the official methods of the Association of Official Agricultural Chemists (*2, pp. 335, 336, 25, 344, 341, 480, 342, and 339*, respectively). Crude fiber was determined by the Kennedy modification (*11*). Pentosans were also determined on a sample treated for weighing as crude fiber and both pentosans and crude protein determined on a fat-free sample which had been extracted for 30 minutes with boiling N/50 sulphuric acid, followed by 15 minutes of boiling after the addition of enough sodium hydroxide to neutralize the acid and give a solution N/50 with regard to the alkali (*7*). Only part of these values are reported in this paper, however their determinations were necessary in order to calculate the values for the nitrogen-free extract.

CALCULATIONS

Pentosans in nitrogen-free extract.—The differences between the total pentosans and the pentosans in crude fiber are taken to be the pentosans in the nitrogen-free extract.

Residual nitrogen-free extract.—The percentages of sugars, starch, and pentosans in the nitrogen-free extract were added together and the sum subtracted from the total nitrogen-free extract. The remainder is termed the residual nitrogen-free extract.

Insoluble pentosans in nitrogen-free extract.—The pentosans were determined on the residue insoluble in N/50 acid and alkali. The pentosans in the crude fiber as determined previously were subtracted, and the remainder is termed the insoluble pentosans in nitrogen-free extract. The soluble pentosans in the nitrogen-free extract were calculated by differences.

Insoluble residual nitrogen-free extract.—From the value for the residue insoluble in N/50 acid and alkali was subtracted the sum of the value for crude fiber plus the values for the crude protein and the pentosans in that insoluble residue. The difference is termed the insoluble residual nitrogen-free extract. The value for the soluble residual nitrogen-free extract was calculated by differences.

DATA

Table 1 gives the composition of the feeds on the moisture-free basis; all values are the average of three determinations. Table 2 records the composition of certain constituents of the nitrogen-free extract calculated as percentages of that fraction.

TABLE 2.—*Composition of the nitrogen-free extract of feeding stubs*

Feed	Uronic acid anhydrides	Sugars and starch	Pentosans			Residual nitrogen—free extract		
			Soluble	Insoluble	Total	Soluble	Insoluble	Total
	Percent	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
Alfalfa hay	25.76	12.01	17.24	8.27	25.51	29.67	32.83	62.50
Corncobs	9.99	6.91	19.20	33.55	52.74	6.13	34.20	40.33
Corn bran	10.63	12.23	41.10	8.92	56.45	18.24	13.08	31.32
Peanut hulls	41.50	14.35	6.87	34.15	40.68	9.59	34.90	44.49
Peanut kernels	12.84	57.93	15.25	6.29	21.54	14.65	5.81	20.46
Rye	.88	72.86	9.77	2.14	11.91	11.37	3.86	15.23
Timothy hay	10.60	12.08	2.61	26.30	28.90	29.75	29.24	58.99
Wheat bran	7.29	23.37	23.61	20.72	44.34	16.23	16.06	32.29
Wheat shorts	2.44	38.04	14.16	7.57	21.74	31.95	8.27	40.22

DISCUSSION

In general the uronic acid content only partially tends to parallel the crude fiber content; in the case of the two hays and the corncobs the values are out of line. The pentosan content of the crude fiber shows a better agreement with the total crude fiber, thus suggesting that the variations in the uronic acid content are to be ascribed to the portion found in the nitrogen-free extract.

In comparing the values obtained for the various constituents in the nitrogen-free extract it is to be recognized that the "pentosan" values will include the pentose moiety of the uronic acid anhydrides. On the basis of equivalent weights 75 percent of the uronic acid anhydride is pentose, but Norris and Resch (*18*) have shown that the uronic acids yield only approximately 42 percent of the theoretical amount of furfural. On this basis 31.5 percent of the weight of the uronic acid anhydrides is included in the "pentosan" fraction as determined. The appropriate deductions from the total pentosan in the nitrogen-free extract have been made to give the corrected pentosan values. Only in the case of the alfalfa hay and the peanut hulls

is the change very great. The corrected pentosan value of the former is 17.41 percent as contrasted with 25.51 percent of the nitrogen-free extract, while in the case of the peanut hulls the value becomes 27.62 percent instead of 40.68 percent. Since it is not known how the uronic acids are distributed as between soluble and insoluble "pentosans", and since the corrections are slight (ranging in difference from 0.3 to 3.3 percent), the corrected values for the other feeds are not shown.

An examination of table 1 fails to reveal any very great regularity between the uronic acid content and the total nitrogen-free extract of the feeds examined, although there is a tendency for the two to vary inversely. As is to be expected, the concentrates contain higher percentages of starch and the soluble sugars, and with the exception of the peanut kernels, these latter vary inversely with the uronic acid content. Among the roughages the starches and sugars occur at about the same lower level but associated with higher concentrations of uronic acids. The proportionalities between the various known constituents of the nitrogen-free extracts are shown more clearly by the method of calculation employed in table 2.

The residual nitrogen-free extract contains the miscellaneous products; of these the uronic acid anhydrides in the feeds are here determined for the first time. There is no marked tendency to regularity between the content of uronic acids and the total residual extract. Except in the case of corn bran and peanut kernels, the uronic acid content of these feeds increases with the insoluble residual nitrogen-free extract, but no regularity appears when the soluble portion is considered.

Since the pentosan fractions as determined should include that arising from the uronic acids present, as pointed out earlier; and since pectins, hemicelluloses, and gums contain uronic acids (*4, 5, 7*); and since the pectins and gums may be sufficiently soluble to escape the crude fiber determination (*5*), we might expect to find some relationship between the uronic acids and pentosans. There is, again, no relationship to be observed when the soluble and the insoluble pentosans are separately compared with the uronic acid content of each feed.

The digestibility of the total nitrogen-free extract is no doubt the resultant of the digestibility of the individual components of this fraction. Investigators, particularly Fraps (*10*), have referred the lower values of the digested matter of roughages as compared with those of concentrates to the presence of unknown and undetermined constituents. Fraps points out that the "pentosans soluble in N/50 acid and alkali are digested to a greater extent than the remaining pentosans" (*7*) and that "the nitrogen-free extract soluble in N/50 acid and alkali is, as a rule, digested to a greater extent than that not soluble" (*8*). With this in mind the calculated percentages of digestibility of the feeds as shown in table 3 were made by assuming that the starches and sugars were 100 percent utilized, and that the pentosans and the residual nitrogen-free extract were utilized to the extent that they were soluble in N/50 acid and alkali. The calculated digestibility was arrived at by giving effect to the proportions of the various constituents. This calculation was made in order to compare the results with those obtained for the nitrogen-free extract from feeding trials as summarized by Henry and Morrison (*12, appendix table II*).

TABLE 3.—*Digestibility of feeds and of their nitrogen-free extract as related to uronic acid content of each*

Feed	Uronic acid content of—		Calculated digestibility of total nitrogen-free extract	Coefficients of digestibility from feeding trials (Henry and Morrison (*12*))
	Feeds	Nitrogen-free extract		
	Percent	*Percent*	*Percent*	
Alfalfa hay	10.40	25.76	58.92	70–72
Corncobs	5.88	9.99	32.24	52
Corn bran	6.64	10.63	71.57	80
Peanut hulls	6.00	41.50	30.81	
Peanut kernels	1.92	12.84	87.83	84
Rye	.72	.88	94.00	88–94
Timothy hay	5.00	10.60	44.44	47–62
Wheat bran	3.88	7.29	63.21	66–72
Wheat shorts	1.68	2.44	84.15	78–88

The calculated digestibilities agree surprisingly well with results obtained by actual feeding trials. There is a closer agreement in the case of the concentrates with their high content of starches and sugars and the lower content of uronic acid and residual nitrogen-free extract. On the other hand, the calculated digestibilities are somewhat lower than those obtained by feeding trials in the case of the roughages which contain a greater amount of uronic acid anhydrides. These latter are only part of the residual nitrogen-free extract, but the results suggest that the lowered digestibility may in part be accounted for by the uronic acids.

SUMMARY

Analyses on nine feeds classified as concentrates, grain byproducts, and roughages have been made in the usual way but to include uronic anhydrides in the residual nitrogen-free extract. Roughages in general contain a higher percentage of the uronic acid anhydrides than do the concentrates. There does not appear to be any regularity between the uronic anhydride content and the other constituents of the nitrogen-free extract. A calculated artificial coefficient of digestibility of these feeds compares fairly well with the results of feeding trials, but the parallelism is less close in the feeds with a high nitrogen-free extract, part of which is of a uronic acid nature.

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THE EFFECT OF MECHANICAL PROCESSING OF FEEDS ON THE MASTICATION AND RUMINATION OF STEERS [1]

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INTRODUCTION

Numerous data are available in the literature regarding the effects which various methods of preparation of concentrates and roughages have on the utilization of these feeds by cattle. The conditions under which the different trials have been conducted are so variable from the standpoint of the type of animal, indices used, and the degree of control of the many factors involved that it is difficult to draw conclusions from the results reported.

If mechanical processing of feeds is of any value other than increasing palatability or decreasing waste, it would appear that it must certainly save the animal some energy in the comminution of the feed by reducing the amount of work required in the processes of mastication and rumination. If this be true, the energy required for the so-called work of digestion would be reduced and the metabolizable energy so conserved should be available for production purposes.

REVIEW OF THE LITERATURE

Bergman and Dukes [2] reported a critical study on two cows fed a normal dairy ration of grain, silage, and hay. They found that one animal spent 6 hours and 42 minutes and the other spent 8 hours and 22 minutes daily in rumination. In the case of each animal this process occurred in 15 to 20 periods which were evenly distributed over the entire day. The length of these periods varied from 2 to 49 minutes with an average of 26 minutes. The total number of boluses regurgitated paralleled the total number of minutes of rumination. Approximately 59 seconds was devoted to each bolus and about 5 seconds of this time was occupied by the processes of rumination other than remastication and reinsalivation. No correlation between the amounts of feed consumed and the time spent in rumination was reported.

Fuller [3] obtained detailed information on three dairy cows fed a normal ration. He reported averages of 5 hours 57 minutes eating, 8 hours 5 minutes ruminating, 53.9 seconds remasticating each bolus, and 3.73 seconds spent in deglutition and regurgitation. Twenty-two cows in stanchions daily spent approximately 3 hours eating, 8 hours ruminating, and made approximately 41,000 jaw movements daily. The rate of the jaw movements depended on the kind of material being chewed—94 per minute for the mastication of grain and silage, 78 per minute for the mastication of hay, and 55 per minute during rumination.

[1] Received for publication Mar. 29, 1937; issued November 1937.

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Schalk and Amadon [4] reported data on the time required for the mastication of various feeds by dairy cows. They found that the following lengths of time were required for 5-pound allowances of the various feeds: Whole oats 10 minutes, corn on the cob 18 minutes, ground feed 14 minutes, hay 15 minutes.

The same authors [4] obtained results of a study of rumination with nine dairy cows on rations of ground grain, alfalfa hay, and corn silage. The animals were followed continuously for 60 hours. The observations obtained, based on a 24-hour period, were 6.14 hours occupied in eating, 6.89 hours ruminating, and 10.90 hours resting.

Four animals were observed continuously for 60 hours for more detailed information,[4] and it was found that the total time spent in rumination was divided into 162 periods including 66 with the animal in the standing position, 75 in the lying position, and 21 in both positions for part of the period. The average number of boluses per period was 25.37, with 50.32 chews being made on each bolus. During the entire period of 60 hours the four animals regurgitated 4,110 boluses and made 206,663 chews.

Two animals were followed in detail for 24 hours while on pasture during the summer.[4] Thirty rumination periods were observed to average 26.1 boluses per period and the average number of chews per bolus was 39.11. Sixteen of these periods occurred while the animals were standing and 14 while lying. They regurgitated 793 boluses and made 31,013 jaw movements during the 24 hours.

The authors concluded that such factors as age, condition of the teeth, and the nature and kind of feed all affected rumination. Flies, mosquitoes, dogs, interference by herd mates, or any condition which would worry, frighten, or disturb the animal may lead to a cessation of rumination. If the stoppage was momentary, the animal would hold the bolus in the oral cavity and presently would resume chewing. However, the number of chews was always lessened and the total time of rumination decreased.

The present investigations were undertaken as part of a larger program on the study of digestion in cattle. They were planned to determine the effects of the processing of both concentrates and roughages on mastication and rumination in steers of various ages.

METHOD OF PROCEDURE

The animals used were ordinary feeder steers which had been purchased on the open market. They had all been operated on to produce rumen fistulas for purposes other than the experiments to be reported. All the steers were in good physical condition but in rather thin flesh during these trials. Their ages at the beginning of the tests varied from 17 to 31 months.

The following rations were fed to each steer:

Ration 1, whole alfalfa hay.

Ration 2, coarsely cut alfalfa hay—modulus 4.55—uniformity 5:5:0.[5]

[4] SCHALK, A. F., and AMADON, R. S. PHYSIOLOGY OF THE RUMINANT STOMACH (BOVINE); STUDY OF THE DYNAMIC FACTORS. N. Dak. Agr. Expt. Sta. Bull. 216, 64 pp., illus. 1928.

[5] The first expression of figures is the modulus of fineness; the second expression is the uniformity of the material in regard to size of particles. The fineness of the material was made by the modulus system. Seven screens are used which range from a ¾-inch mesh to 100 meshes per inch. This series of screens separates the material into various particle sizes. By weighing what remains on each screen a figure is computed which gives the fineness of the material. The larger number denotes coarse material and the smaller number denotes fine material. The consistency of uniformity of the material was arrived at by adding the percentage of material remaining on the coarse screens, the medium screens, and the fine screens. This gives a ratio of three figures which denotes the relationship in the amounts of coarse, medium, and fine material in any given sample.

Ration 3, finely cut alfalfa hay—modulus 3.55—uniformity 1:7:2.
Ration 4, ground alfalfa hay—modulus 3.25—uniformity 1:6:3.
Ration 5, whole alfalfa hay, protein supplement—modulus 2.01—uniformity 0:3:7, shelled yellow corn—modulus 6.01—uniformity 10:0:0.
Ration 6, whole alfalfa hay, protein supplement—modulus 2.01—uniformity 0:3:7, ground shelled yellow corn—modulus 3.56—uniformity 2:6:2.
Ration 7, protein supplement—modulus 2.01—uniformity 0:3:7; shelled yellow corn—modulus 6.01—uniformity 10:0:0.

A good-quality alfalfa hay was used throughout the trials. The coarsely cut and finely cut hays were approximately 2-inch and ¼-inch pieces which had been cut in an ordinary hay cutter. Figure 1 shows samples of the alfalfa hay as fed. The hay and corn were ground by a hammer-type mill. The protein supplement consisted of a mixture

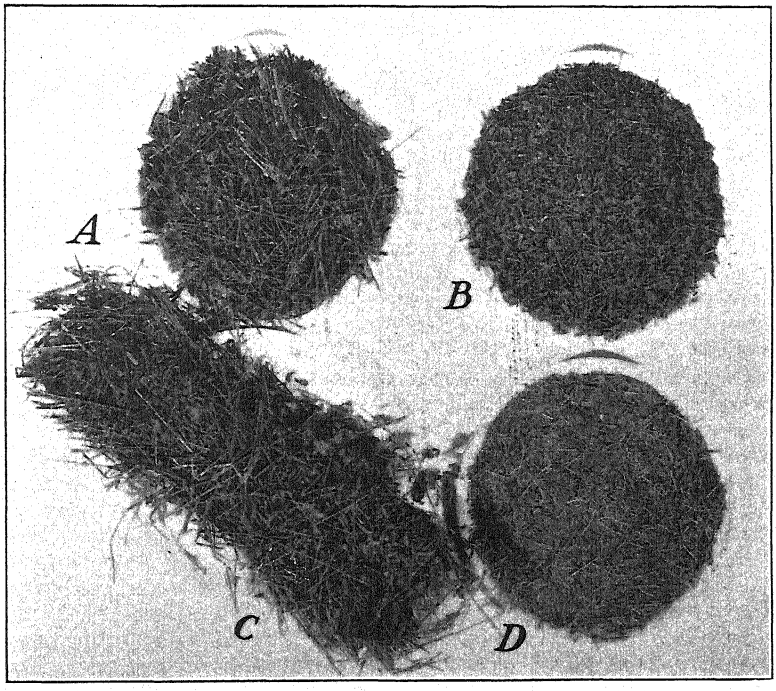


FIGURE 1.—Samples of the various hays fed: *A*, coarsely cut alfalfa, approximately 2-inch lengths; *B*, finely cut alfalfa, approximately ¼-inch lengths, *C*, whole alfalfa; *D*, ground alfalfa.

of dry-rendered tankage, cottonseed, linseed, and soybean-oil meals, steamed bonemeal, limestone, and salt. Block salt was before the animals at all times.

The rations were fed at approximately 8 a. m. and 4 p. m. in such amounts as the animals would consume readily. Each steer was given exactly the same amount of the various kinds of hay in order to have a common basis on which to compare the amounts of mastication and rumination. The corn and hay of the mixed rations were always fed in the ratio of 3 parts of corn to 1 part of hay, the amount of corn being regulated by the appetite of the animal. The shelled corn in ration 5 was replaced by an equivalent weight of ground corn in ration 6. In the last trial (ration 7), the roughage was omitted from the feed, the

amount of protein supplement was maintained as in the two previous trials, and the animals were allowed all the shelled corn they would consume.

A preliminary period of a week, in some cases more, was introduced between trials, during which time the animals were fed the rations to be tested during the following period. This preliminary period served to accustom the animals to the new feed and also to remove any residue of the preceding ration which might remain in the rumen or reticulum.

Each trial was begun at approximately 8 a. m. when the animals were given their morning feed. The number of jaw movements was recorded by means of mechanical counters and the time taken was noted. This process was repeated about 4 p. m., when the evening feed was offered. During the remainder of the 24-hour period the steers were under constant surveillance, and the time, number of chews, number of boluses regurgitated, and the number of chews per bolus during the entire daily rumination were recorded. Each trial was continued for 48 consecutive hours, and the data presented represent, in each case, an average of two 24-hour periods.

OBSERVATIONS

In general, as the finer hay was fed the number of chews taken for mastication was reduced in all the animals regardless of age (table 1). There was no apparent difference in the amount of mastication required for the long and the coarsely cut hay in the case of the youngest steer (A). Steer D did not relish the ground hay and minced about while eating, which would account for the increased number of jaw movements taken for the mastication of this material. All of the animals took more chews in the mastication of ground corn than shelled corn. The number of chews per unit weight of feed decreased as the age of the animal and the amount of feed consumed increased.

The total time spent by each steer in the mastication of the different rations (fig. 2) varied directly with the total number of chews made. When the time required per unit of feed was calculated it was apparent that the younger animals (A and E) spent more time in masticating their feed than the older animals (B and D). The percent of the daily time spent in mastication varied from 4.3 to 11.0 depending on the fineness of the material eaten. In the case of the various roughages, the amount of time decreased as the size of the particles decreased. The reverse was true in the case of the corn, shelled corn requiring less time for mastication than did ground corn. The only obvious reason for the discrepancy in the time required for the mastication of whole alfalfa when fed alone and in combination with the concentrates is the fact that on the higher plane of nutrition the appetites of the animals were keener. The rate of chewing was variable but could not be correlated with the physical condition of the feed.

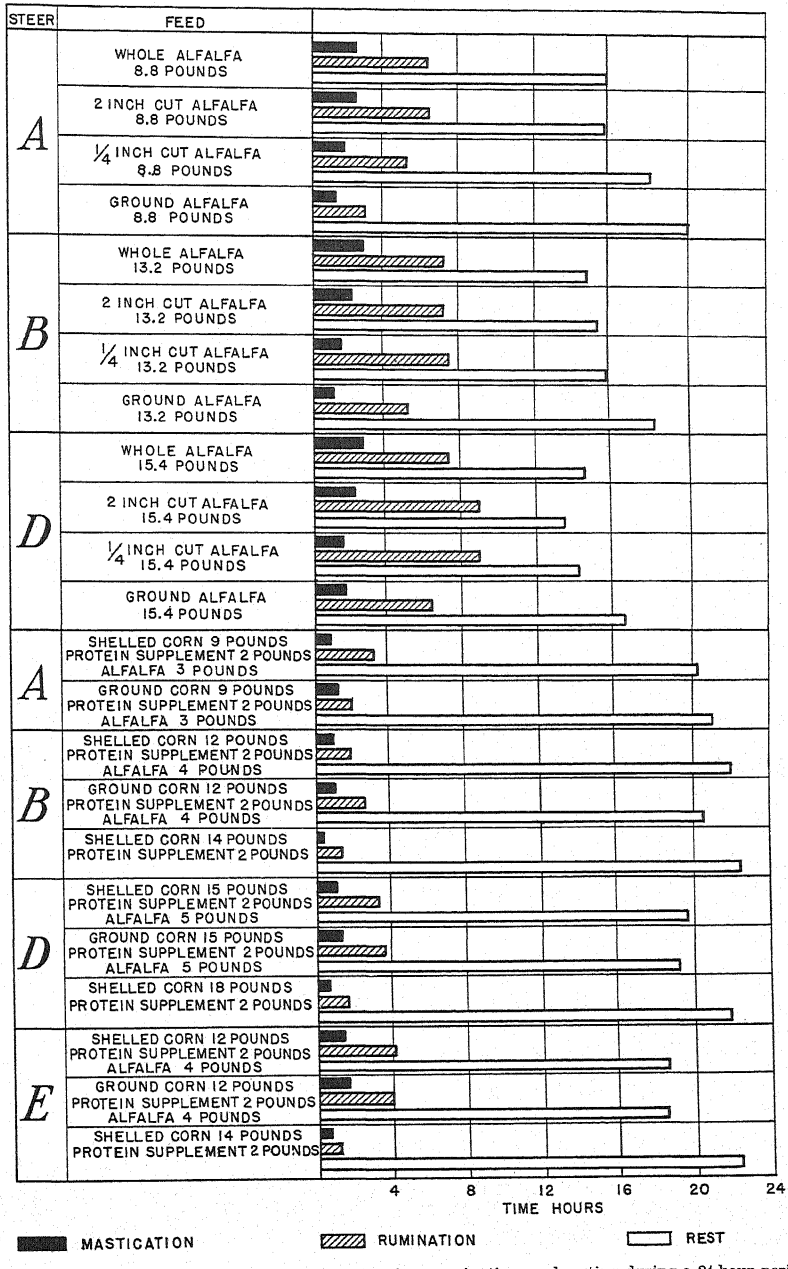


FIGURE 2.—Total time spent by steers in masticating, ruminating, and resting during a 24-hour period. Age of animals: A, 17 to 28 months; B, 22 to 33; D, 31 to 42; and E, 28 months

TABLE 1.—*Effect of various methods of preparation of alfalfa hay and of grinding corn on mastication by steers*

[Data are for two 24-hour periods]

Steer	Age	Quantity and preparation of ration	Mastication					
			Chews	Chews per pound	Total time	Time per pound	Portion of day spent in mastication	Chews per minute
	Months		*Number*	*Number*	*Minutes*	*Minutes*	*Percent*	*Number*
		ALFALFA HAY						
A	17	8.8 pounds whole alfalfa	10,298	1,170	141	16.0	9.8	73
		8.8 pounds 2-inch cut alfalfa	10,686	1,214	143	16.3	9.9	75
	19	8.8 pounds ¼-inch cut alfalfa	6,737	766	99	11.3	6.9	68
		8.8 pounds ground alfalfa	5,609	637	76	8.6	5.3	74
B	22	13.2 pounds whole alfalfa	13,085	991	159	12.0	11.0	82
		13.2 pounds 2-inch cut alfalfa	10,280	779	121	9.2	8.4	85
	24	13.2 pounds ¼-inch cut alfalfa	6,182	468	84	6.4	5.8	74
		13.2 pounds ground alfalfa	4,556	345	62	4.7	4.3	73
D	31	15.4 pounds whole alfalfa	13,935	905	158	10.3	11.0	88
		15.4 pounds 2-inch cut alfalfa	9,824	638	126	8.2	8.8	78
	33	15.4 pounds ¼-inch cut alfalfa	7,060	458	90	5.8	6.3	78
		15.4 pounds ground alfalfa	8,330	541	96	6.2	6.7	87
		GROUND CORN						
A	28	3 pounds whole alfalfa, 2 pounds protein supplement, 9 pounds shelled corn	1,723	574	26	8.7	1.8	66
			2,031	185	26	2.4	1.8	78
	28	3 pounds whole alfalfa, 2 pounds protein supplement, 9 pounds ground corn	2,054	685	28	9.3	1.9	73
			2,667	242	36	3.3	2.5	74
B	36	4 pounds whole alfalfa, 2 pounds protein supplement, 12 pounds shelled corn	2,643	661	35	8.8	2.4	76
			1,721	123	20	1.4	1.4	86
	36	4 pounds whole alfalfa, 2 pounds protein supplement, 12 pounds ground corn	2,423	606	32	8.0	2.2	76
			2,232	159	25	1.8	1.7	89
	36	2 pounds protein supplement, 14 pounds shelled corn	1,872	117	22	1.4	1.5	85
D	42	5 pounds whole alfalfa, 2 pounds protein supplement, 15 pounds shelled corn	2,719	544	38	7.6	2.6	72
			2,429	143	30	1.8	2.1	81
	42	5 pounds whole alfalfa, 2 pounds protein supplement, 15 pounds ground corn	2,850	570	39	7.8	2.7	73
			3,159	186	36	2.1	2.5	88
	42	2 pounds protein supplement, 18 pounds shelled corn	3,012	151	37	1.9	2.6	81
E	28	4 pounds whole alfalfa, 2 pounds protein supplement, 12 pounds shelled corn	3,653	913	53	13.2	3.7	69
			2,202	157	33	2.4	2.3	67
	28	4 pounds whole alfalfa, 2 pounds protein supplement, 12 pounds ground corn	3,987	997	51	12.7	3.5	78
			3,400	243	42	3.0	2.9	81
	28	2 pounds protein supplement, 14 pounds shelled corn	2,699	169	36	2.3	2.5	75

The effects of processing feed on rumination are presented in table 2. Cutting the alfalfa into lengths as short as one-fourth inch had no marked effect on the number of chews, the time taken for rumination (fig. 2), the number of periods this time was divided into (fig. 3), the number of boluses regurgitated, or the number of chews made and the time spent on each bolus. However, grinding the hay reduced the number of chews and the time required for rumination and this

reduced the number of chews made per pound of feed. The daily time spent in rumination of the whole and cut hays averaged approximately 29 percent, while in the case of the ground hay this was reduced to 19 percent. The youngest animal (A) evidenced a slight reduction in the number of chews and the time required for rumination on the

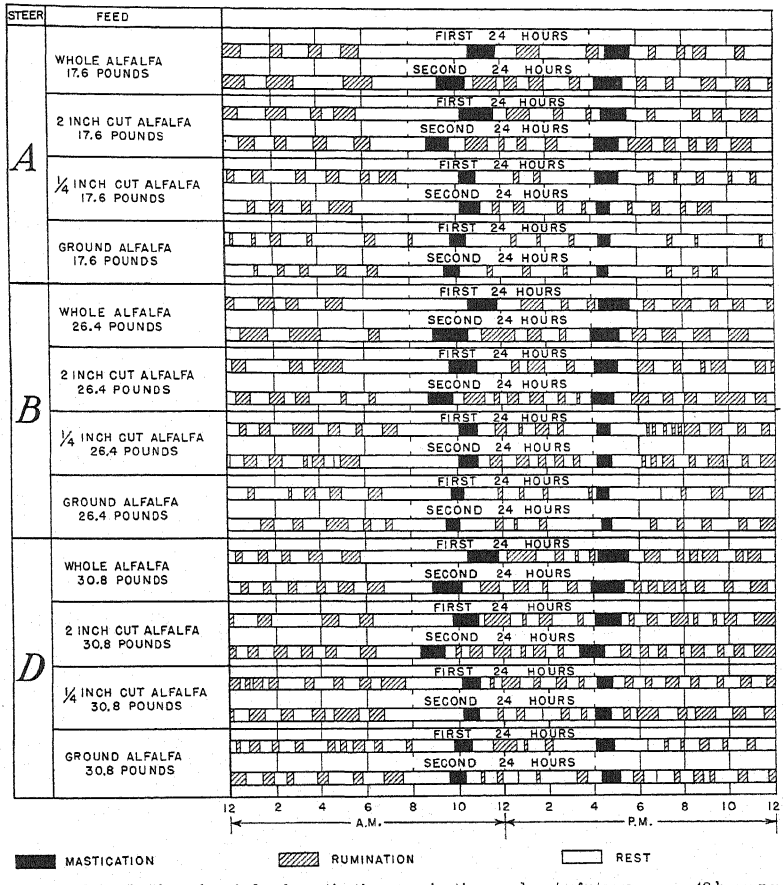


FIGURE 3.—Distribution of periods of mastication, rumination, and rest of steers over a 48-hour period
Age of animals: A, 17 to 19 months; B, 22 to 24 months; and D, 31 to 33 months.

finely cut hay, but these differences were small and could only be regarded as an indication.

The mixed rations (5, 6, and 7) required fewer chews and less time for rumination (table 2) than the rations consisting of hay alone. The differences due to grinding the corn were not very consistent, but when the data from all the steers were averaged no effect was demonstrated. However, the removal of the roughage from the ration reduced the rumination process to a minimum as measured by the time and the number of chews taken.

TABLE 2.—*The effect of various methods of preparation of alfalfa hay and of grinding corn on rumination by steers*

[Data are for two 24-hour periods]

Steer	Age	Quantity and preparation of ration	Rumination											
			Chews	Chews per pound	Total time	Time per pound	Portion of day spent ruminating	Boluses	Boluses per pound	Chews per bolus	Periods	Time per period	Time per bolus	Chews per minute
	Months	ALFALFA HAY	*Number*	*Number*	*Minutes*	*Minutes*	*Percent*	*Number*	*Number*	*Number*	*Number*	*Minutes*	*Seconds*	*Number*
A	17	8.8 pounds whole alfalfa	19,043	2,164	363	41.3	25.2	350	39.8	54.4	10.5	34.6	62.2	52
		8.8 pounds 2-inch cut alfalfa	19,951	2,267	371	42.2	25.8	365	41.5	54.7	12.0	30.9	61.0	54
	19	8.8 pounds ¼-inch cut alfalfa [1]	15,745	1,789	297	33.8	20.6	312	35.5	50.5	13.5	22.0	57.1	53
		8.8 pounds ground alfalfa [1]	7,536	856	166	18.9	11.5	191	21.7	39.5	12.0	13.8	52.1	45
B	22	13.2 pounds whole alfalfa	22,467	1,702	418	31.7	29.0	488	37.0	46.1	10.5	39.8	51.4	54
		13.2 pounds 2-inch cut alfalfa	22,506	1,705	415	31.4	28.8	543	41.1	41.4	13.5	30.7	45.8	54
	24	13.2 pounds ¼-inch cut alfalfa [1]	22,173	1,680	427	32.3	29.7	533	40.4	41.6	26.0	20.4	48.1	52
		13.2 pounds ground alfalfa [1]	14,867	1,126	299	22.7	20.8	360	27.3	41.3	13.0	23.0	49.8	50
D	31	15.4 pounds whole alfalfa	22,702	1,474	426	27.7	29.6	468	30.3	48.5	15.5	27.5	54.6	53
		15.4 pounds 2-inch cut alfalfa	24,696	1,604	524	34.0	36.4	514	33.4	48.0	17.0	30.8	61.2	47
	33	15.4 pounds ¼-inch cut alfalfa [1]	23,362	1,517	517	33.6	35.9	500	32.5	46.7	17.5	29.5	62.0	45
		15.4 pounds ground alfalfa [1]	15,723	1,021	366	23.8	25.4	365	23.7	43.1	18.0	20.3	60.2	43
		GROUND CORN												
A	28	3 pounds whole alfalfa, 2 pounds protein supplement, 9 pounds shelled corn	7,615	544	187	13.4	13.0	282	20.1	27.0	12.5	15.0	39.8	41
	28	3 pounds whole alfalfa, 2 pounds protein supplement, 9 pounds ground corn	3,956	283	110	7.9	7.6	125	8.9	31.6	10.0	11.6	52.8	36
B	36	4 pounds whole alfalfa, 2 pounds protein supplement, 12 pounds shelled corn	4,527	252	105	5.8	7.3	130	7.2	34.8	8.0	13.1	48.5	43
	36	4 pounds whole alfalfa, 2 pounds protein supplement, 12 pounds ground corn	7,228	402	155	8.6	10.8	181	10.1	40.0	9.0	17.2	51.4	47
	36	2 pounds protein supplement, 14 pounds shelled corn	2,923	183	80	5.0	5.5	85	5.3	34.4	8.0	10.0	56.5	37
D	42	5 pounds whole alfalfa, 2 pounds protein supplement, 15 pounds shelled corn	8,673	394	194	8.8	13.5	200	9.1	43.4	14.0	13.9	58.2	45
	42	5 pounds whole alfalfa, 2 pounds protein supplement, 15 pounds ground corn	9,817	446	213	9.7	14.8	217	9.9	45.2	14.5	14.7	58.9	46
	42	2 pounds protein supplement, 18 pounds shelled corn	4,066	203	96	4.8	6.7	112	5.6	36.3	8.5	11.3	51.4	42
E	28	4 pounds whole alfalfa, 2 pounds protein supplement, 12 pounds shelled corn	10,389	577	246	13.7	17.1	250	13.9	41.6	11.0	22.4	59.0	42
	28	4 pounds whole alfalfa, 2 pounds protein supplement, 12 pounds ground corn	10,126	563	237	13.2	16.5	293	16.3	34.6	14.0	16.9	48.5	43
	28	2 pounds protein supplement, 14 pounds shelled corn	2,120	133	65	4.0	4.5	63	4.0	33.7	5.5	11.8	61.9	33

[1] Several regurgitations before bolus was swallowed.

The total number of boluses regurgitated, the number of chews, and the time spent on each bolus were practically the same when the rations consisted of cut hays or whole hay, but when ground hay or the mixed rations were fed, these indices were greatly reduced, with the exception that the time spent on each bolus was fairly constant on all the rations. Feeding a ration devoid of roughage resulted in a minimum number of boluses being regurgitated and a slight reduction in the number of chews per bolus as compared to the other mixed rations. A peculiar observation was made when the animals were

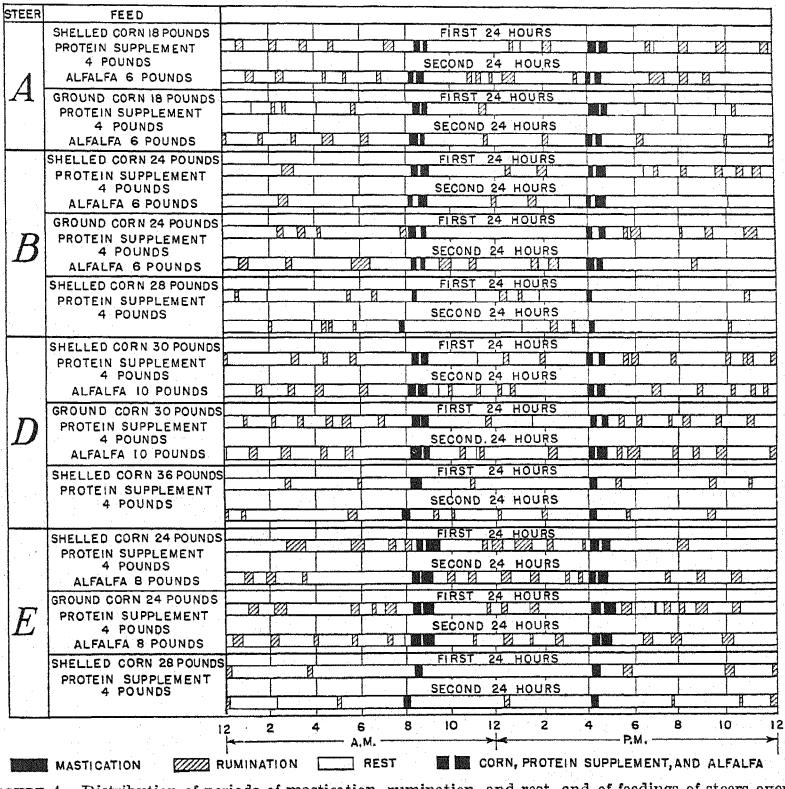


FIGURE 4.—Distribution of periods of mastication, rumination, and rest, and of feedings of steers over a 48-hour period. Age of animals: A, 28 months; B, 33; D, 42; and E, 28 months.

fed the finely ground hay and the mixed rations. Two or three, sometimes as many as six, boluses were regurgitated and remasticated before any of the material in the oral cavity was swallowed.

The rumination process was divided into a number of periods scattered throughout the 24 hours (figs. 3 and 4). The processing of both roughage and concentrates had no apparent effect on the number or distribution of these periods, but it is obvious from the graphs that the length of these periods was reduced materially when ground hay or the mixed rations were fed.

The rate of chewing was practically constant for the whole and cut hays and showed only a slight reduction on the ground alfalfa. However, the rate on the mixed rations appeared to be slower than whe..

roughage alone was fed, and this was further reduced by the entire removal of the roughage from the ration.

DISCUSSION

The object of the mastication of feed is apparently twofold—the comminution of the feed particles and the incorporation of the saliva in the feed. These two processes occur simultaneously and which of them is predominant depends upon several factors. One of the more important of these factors is the physical condition of the feed. In every case except that of Steer A the data show a reduction in the number of chews per pound of feed required for the mastication of alfalfa as the particles became smaller. An average of approximately twice as many chews were made on the whole alfalfa as on the ground alfalfa. This would naturally be expected since comminution of the feed must necessarily have been more important in preparing the whole hay for deglutition than the ground hay. It was actually observed when whole hay was fed that prehension was much slower and the chewing more vigorous and deliberate than when the processed hays were eaten. An average of approximately four times as many chews were made in masticating a pound of whole alfalfa as were necessary for a pound of whole shelled corn and protein supplement, while the number of chews required for ground corn was approximately 25 percent greater than for shelled corn.

All of the observed results may be explained on the basis of the primary object of the mastication of the various rations. When the comminution process was the primary object, as in the case of the whole hay, the maximum number of chews per unit of feed resulted. The insalivation process, taking place simultaneously, was not a limiting factor in the time or number of chews required. As the hay was processed to reduce the size of particles, the comminution process required less time and energy, and therefore the time and the number of chews were reduced. This was true within certain limits. When the hay was finely ground the insalivation process had attained predominance, and the time and energy required were apparently dependent upon this factor.

When shelled corn was fed, prehension was extremely rapid and the corn was swallowed as soon as it was sufficiently covered with saliva to allow easy deglutition. Apparently the reduction in the size of particles was not a factor of major importance in the mastication of this material. This was also true in the case of ground corn, and it appears as though the increased number of chews can only be explained on the basis of the difficulty of insalivation. Shelled corn kernels, protected by their outer coating high in fiber, are not capable of absorbing as much saliva as the soft, starchy endosperm which is exposed by the ground corn. Therefore, it is apparent that it will be more difficult to prepare the ground corn for deglutition, and this explains the increased number of chews and length of time required.

The age of the animal would seem to affect mastication. The younger animals took more chews per unit weight of feed than the older steers.

Other factors which probably affect the mastication process are metabolic rate, general physical condition, and appetite. It is always difficult to measure such factors and evaluate their effects, but some ...ication of their collective effect exists in the data presented. When

the animals were on the higher plane of nutrition of the mixed rations they were in better general physical condition, gained in body weight, and had much keener appetities than when the ration consisted of alfalfa hay alone. The animals spent less time and made fewer chews in the mastication of the mixed ration in spite of the fact that they actually consumed more total feed. Just how much of this effect can be attributed to the factors mentioned is difficult to say, but it seems plausible at least to assume that they played some part in the change.

The most important factor in the amount of rumination observed in the steers was the quantity of roughage in the ration. When rations composed of corn, protein, and hay were fed the number of chews during rumination was approximately 60 percent fewer than when alfalfa was fed alone. This decrease occurred in spite of the fact that the mixed rations contained more pounds of feed than were fed as alfalfa alone. The removal of all roughage from the ration reduced the amount of rumination to a minimum, which was approximately 45 to 50 percent of that occurring on the mixed ration and approximately 15 percent of that on the rations of whole or cut hay. When the ration contained no roughage rumination was slow and listless and had the appearance of being done from an inherent impulse rather than from any desire or necessity. No ill effects were noted on the general health or appetite of the animals when the ration consisted of corn and protein supplement for a period of 2 weeks.

Cutting hay had no effect on the amount of rumination. Since the comminution of the feed particles is usually considered a major object of rumination, it would appear that the differences in the particle size had been minimized by the differences in the amounts of mastication already noted on the different hays. Another possibility is the reduction of the particles in the rumen due to such processes as maceration caused by the motility of the rumen walls, bacterial fermentation, or chemical reactions, which undoubtedly occur continuously. The feeding of ground hay resulted in a 40-percent reduction in rumination. It appeared from observation that reinsalivation was the predominant factor since the chewing was not vigorous and several boluses were regurgitated before deglutition occurred.

Grinding shelled corn had no apparent effect on the amount of rumination.

SUMMARY AND CONCLUSIONS

From the results obtained, it would appear that cutting hay for steers might conserve some metabolizable energy because of a reduction in the energy required for mastication, but no economy was effected because of a reduction in rumination.

Ground hay required less work for both mastication and rumination.

No economy of metabolizable energy was apparent from a decrease in rumination when shelled corn was ground, and grinding actually increased the work required for mastication.

An important factor in the amount of mastication and rumination required on the rations studied was the proportion of grain and roughage in the ration.

Mastication required from 1.4 to 11.0 percent and rumination required from 4.5 to 36.4 percent of the daily time, depending on the ration.

RELATION OF WATER-SOAKED TISSUES TO INFECTION BY BACTERIUM ANGULATUM AND BACT. TABACUM AND OTHER ORGANISMS [1]

By JAMES JOHNSON

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INTRODUCTION

The epidemiology of certain leaf spot diseases of tobacco and other plants is still the subject of much speculation, even though distinct advances in the knowledge of the subject have been made in recent years. Blackfire or angular leaf spot of tobacco, caused by *Bacterium angulatum* Fromme and Murray, is, for example, a widespread and often a serious disease of this crop. Nevertheless, artificial inoculation with this pathogen under ordinarily favorable conditions of environment for infection with many parasites either fails to produce any symptoms, or, at most, produces only small incipient lesions not comparable to the severe necrosis which often develops under field conditions. This situation remained obscure until recently, when it was shown by Clayton (*3*)[2] that the highly necrotic or "epidemic" form of wildfire (*Bact. tabacum* Wolf and Foster) and blackfire is dependent upon the tissues becoming water-soaked by rain followed by the infection of these areas by the pathogen. The water-soaking explanation of infection, as contrasted with the older conception of leaf wounding by rain as a predisposing factor, marked a distinct step in the understanding of certain diseases of tobacco, particularly the blackfire disease. The experimental method in the present investigation differs from that used by Clayton in that water soaking was induced by internal rather than external water pressure.

The demonstration of the relation of water-soaked tissues to infection by organisms and development of disease now presents new problems on the nature of parasitism and of predisposition and susceptibility of plants to disease. In a practical sense, it also raises questions as to the reliance that can be placed on certain types of control measures such as sanitation and eradication. It will be shown in this paper, for example, that water soaking permits infection of normally immune plant species with various bacterial pathogens, and to some degree with bacteria which are not normally pathogenic. The determination of the host range of an organism, and even the definition of parasitism, may become difficult under such circumstances.

METHODS AND MATERIALS

The chief modification of method used in the present studies beyond those commonly employed or previously described consisted essentially of water soaking the plant tissues by means of the application of a high water pressure to the root system or the cut stem end of plants. The principle involved was perhaps first used by De Bary, and has been

[1] Received for publication May 24, 1937; issued November 1937. Cooperative investigations of the Wisconsin Agricultural, Experiment Station and the Division of Tobacco and Plant Nutrition, Bureau of Plant Industry, U. S. Department of Agriculture.

[2] Reference is made by number (italic) to Literature Cited, p. 618.

frequently employed since in physiological investigations. The apparatus as used in this laboratory was briefly described in 1924 in connection with plant virus studies (*6*). This equipment consisted essentially of a brass container connection to the pipe line of an ordinary water supply under pressure, together with the necessary valves and drain. This container is fitted with a neck to hold a no. 4 rubber stopper, which is securely held in place by means of a packing box similar to that used around valve stems. Rubber stoppers with holes of different sizes were made to hold plant stems of different sizes. These stoppers may be split down one side, so as to slip readily around the stem of the plants. A split brass washer on the top of the stopper permits the packing-box nut to be screwed down sufficiently by hand without displacement of the stopper. The contact around the plant stem may be quickly and almost perfectly made in this manner and will stand 100 pounds of water pressure without leaks (fig. 1).

The soil was washed off the roots before the root system was placed under pressure. If desired, the root system need not be used, the cut end of the stems only being placed under pressure. Water soaking in some plants starts very quickly, and may cover 50 to 100 percent of the leaf area in 15 minutes. With other species or with individual plants 30 to 60 minutes of exposure may be required to secure a limited amount of water soaking. After the plants were water-soaked to the desired degree, they were atomized with a water suspension of the organism to be tested, then removed from the apparatus and the roots or stem ends placed in a flask of water. The plants were then placed in a chamber with a moisture-saturated atmosphere, where the water-soaked conditions could often be maintained for several days if desired. Evident symptoms usually developed after 18 to 48 hours in the moist chamber, but in cases of uncertain infection or none the plants were left in the moist chamber for as long as 4 days.

Tomatoes (*Lycopersicum esculentum* Mill.) were frequently used as the test plant for parasitism because of the ease with which they were handled and water-soaked, but tobacco was used regularly, particularly in connection with the symptom studies on the blackfire and wildfire organisms. Aside from species of Solanaceae, the other plants used were usually chosen at random from species available at the time in the greenhouse or garden.

The organisms used in the experiments were secured from authoritative sources, largely from workers actively engaged in studies of the organism in the Departments of Plant Pathology or Agricultural Bacteriology at the University of Wisconsin.[3]

EXPERIMENTAL RESULTS

The infection experiments have been conducted for the most part without any special consideration to tobacco (*Nicotiana tabacum* L.) as the typical host to *Bacterium angulatum*. It may be recalled that *Bact. tabacum*, the causal organism of wildfire, was reported as possessing a wide host range (*8*) but that according to Clayton (*2*) only species of *Nicotiana* should be regarded as true hosts. Since the close relationship of *Bact. tabacum* and *Bact. angulatum* has come to be accepted

[3] Thanks are due to several associates for furnishing these cultures, and especially to A. J. Braun, who maintained suitable cultures of *Bacterium angulatum* and *Bact. tabacum* for these studies.



FIGURE 1.—The equipment used for water soaking plant tissues by means of a high water pressure on the root system or cut stems of plants. The packing-box nut holding the split rubber stopper is shown at the base of the stem.

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(*1*, *9*), and the relation of water soaking to infection has been established, the host range of one or both of these organisms will no doubt need reconsideration. If we should limit the true hosts to those only on which infection may be secured artificially by ordinary spraying inoculation, together with those which are found to be infected under natural conditions, we should even then possibly need to consider the true hosts of *Bact. angulatum* and *Bact. tabacum* as extending beyond the genus *Nicotiana*. Manifestly, the influence of both the internal and external water relations of the host may be considered in host-range determinations.

INFECTION WITH BACTERIUM ANGULATUM WITHOUT WATER SOAKING

Some of the conditions aside from external environment which favor infection of tobacco with *Bacterium angulatum* have been frequently suggested, among these being the nutritional balance and height of topping of the crop (*4*), but these factors according to Clayton are not clearly separable from the modified susceptibility of the plants to water soaking (*3*).

The writer's experiments, first carried on with *Nicotiana tabacum* (var. Wisconsin Havana Seed) under greenhouse conditions and without water-soaked tissues, were often made unreliable because of the failure to secure infection in sufficient degree to form a satisfactory basis for interpretation of results. *N. tabacum* is relatively very resistant to *Bacterium angulatum* as compared with *N. glutinosa* L. (fig. 2). For example, a group of 46 inoculations to tobacco under a wide range of temperature (10° to 40° C.) and relative humidity (50 to 100 percent) conditions yielded only 6 plants definitely infected, whereas out of 54 *N. glutinosa* plants under the same conditions, 51 were definitely infected. It is significant that distinct infections on *N. glutinosa* were secured at both the temperature and humidity extremes, and that the atmospheric environment following spray inoculation on the whole appeared to play but a minor role in the amount of infection which developed. Pretreatment of *N. tabacum* for 12 hours or more in a humid atmosphere before inoculation was somewhat more reliable and effective in favoring infection than was the treatment after inoculation.

Repeated spray inoculations in the greenhouse to both *Nicotiana tabacum* and *N. glutinosa* on 27 soils (including soil from all tobacco-growing sections of the United States) differing widely in physical structure and chemical fertility yielded no results which could be definitely correlated with soil type, soil fertility, or rate and vigor of growth of the host. In 10 trials performed at different times, however, it was fairly evident that certain 2 or 3 individual soils regularly yielded plants considerably more susceptible to infection than did other soils, the reason for which could not be determined. Still other modifications, such as inoculation by wiping *Bacterium angulatum* over the leaf surface with cheesecloth so as to break the trichomes, failed to yield good infection. The result of all experiments of this type in the absence of water soaking was the failure to secure at will the large angular or blotchy necrotic symptoms characteristic of heavy field infection on ordinary tobacco. However, good infection of the small necrotic or incipient type may be fairly easily secured on nearly all species of *Nicotiana* (*N. repanda* is most resistant or immune) and on species of several other genera with *Bact. angulatum* in the absence of

water soaking. The following species in particular often yielded fair infection: Tomato (*Lycopersicum esculentum* Mill.), potato (*Solanum tuberosum* L.), pepper (*Capsicum annuum* L.) (fig. 3), jimsonweed (*Datura stramonium* L.), apple-of-Peru (*Nicandra physaloides* (L.), Pers.), cucumber (*Cucumis sativus* L.), and pokeweed (*Phytolacca decandra* L.). Other solanaceous species such as black nightshade (*S. nigrum* L.), eggplant (*S. melongena* L.), and physalis (*Physalis pubescens* L.) failed to yield even minute amounts of infection under the above experimental conditions. It is of some interest to note

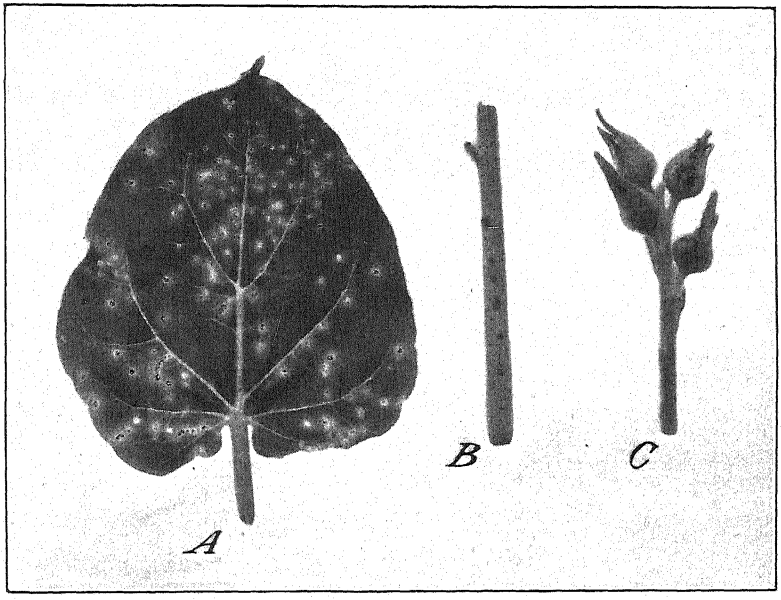


FIGURE 2.—Lesions of *Bacterium angulatum* on leaves (*A*), stems (*B*) and pods (*C*) of *Nicotiana glutinosa*, resulting from spraying inoculation without water soaking. This species is more susceptible than *N. tabacum*, and was frequently used in the trials with ordinary inoculation methods.

that the sterile hybrid of *N. tabacum* (resistant)×*N. glutinosa* (susceptible) is distinctly intermediate in reaction to *Bact. angulatum*.

INFECTION WITH BACTERIUM TABACUM WITHOUT WATER SOAKING

The accumulated experience and observation over a period of years indicates that *Bacterium tabacum* is a more virulent parasite than is *Bact. angulatum*, though admittedly comparative morphological, physiological, and serological studies of the two organisms indicate their close relationship (*1*). The toxin-producing ability of *Bact. tabacum* is, of course, a major difference, and Clayton (*3*) has come to the conclusion from his water-soaking experiments that this is the chief difference between the two organisms as far as infection and pathogenicity are concerned.

Observations in the seedbed and in the field have led the writer to conclude that water soaking is by no means as essential for infection and severe expression of disease with *Bacterium tabacum* as with *Bact. angulatum*. Inoculation of tobacco in the greenhouse by mild spray-

ing has also regularly shown greater virulence in *Bact. tabacum* as measured by the number of infections which develop and the size of the necrotic area. In seeking further experimental proof of this difference, tobacco leaves were inoculated by gently wiping bacterial suspensions from cultures over the leaf surface with cheesecloth, thus breaking the leaf hairs and perhaps occasionally the cuticle. When inoculations are made in this manner *Bact. tabacum* yields heavy infection both as regards number of infections and subsequent necrosis, whereas *Bact. angulatum* yields little or none. Since it might be argued that the wildfire toxin from the culture, and not the bacteria, is responsible for the resultant symptoms, the bacteria were centrifuged out of the suspension in three changes of water, thereby removing the toxin. Wiping inoculation with the bacteria alone, evidently free from perceptible amounts of toxin, yielded almost equally numerous though slower infections (fig. 4, *A*). On the other hand, when the bacteria were removed by heat (the toxin according to Clayton (*2*) being thermostable) and the same method of inoculation employed, there were no symptoms of disease.

These results seem to indicate that, given a favorable external environment of reasonable duration, especially as regards moisture, *Bacterium tabacum* is quite able to enter the cells through wounds, or to enter the stomata and produce lesions of considerable size; whereas *Bact. angulatum* may fail completely to infect under like conditions. Furthermore, this greater virulence does not seem necessarily to be connected with the toxin-producing property of *Bact. tabacum*. It is by no means contended, however, that water soaking does not greatly facilitate infection and the rate of progress of *Bact. tabacum* in the tissues, resulting in extensive necrotic areas, though it seems clear from later experiments that the toxin itself is not of any particular advantage in causing such necroses of the tissues.

WATER SOAKING WITH ARTIFICIAL INTERNAL WATER PRESSURE

The physiology of water soaking of the intercellular spaces of plant tissues by water pressure is not sufficiently understood as yet to warrant extensive discussion. Some species water-soak much more readily than others, e. g., tomatoes much more readily than tobacco. Great differences in individual plants of one variety grown under like conditions may exist, and this difference seems to bear comparatively little relation to the vigor of the plant. A very stunted yellow and red slow-growing tomato, for example, may water-soak quite as easily as a larger, vigorous, rapidly growing plant.

Tomatoes are the most convenient and most susceptible plants with which the writer has worked in infection trials on internally water-soaked tissues. For that reason they have invariably been used as control plants when other species were inoculated to verify both the pathogenicity of the culture used and the favorableness of the subsequent environment under which the plants were placed. Control tomato plants not water-soaked but inoculated were also invariably used in each separate trial.

The greatest interest in connection with *Bacterium angulatum* naturally centers around ordinary tobacco as the host. Comparatively young plants with 8 to 10 leaves but with somewhat elongated internodes (which may be induced by crowding of plants on the bench)

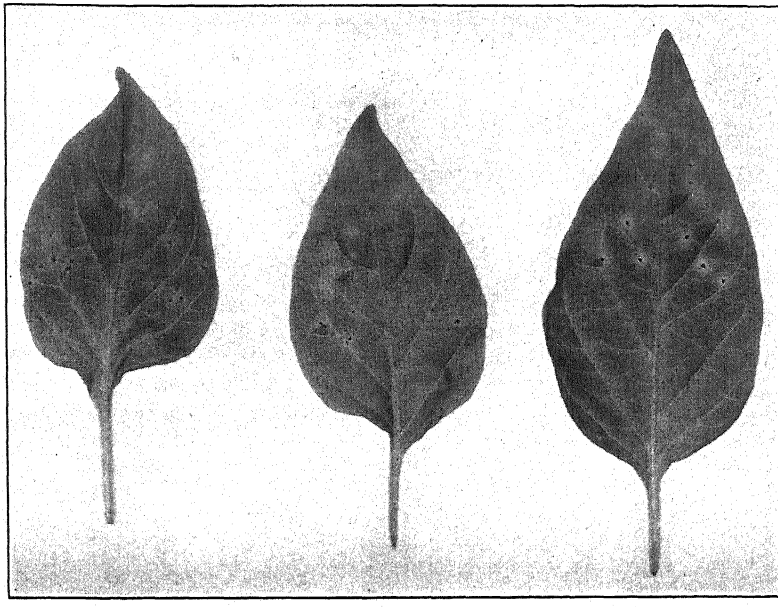


FIGURE 3.—Lesions of *Bacterium angulatum* on pepper secured by ordinary inoculation methods. Many other slightly susceptible hosts outside the Nicotianas have been demonstrated by the same method.

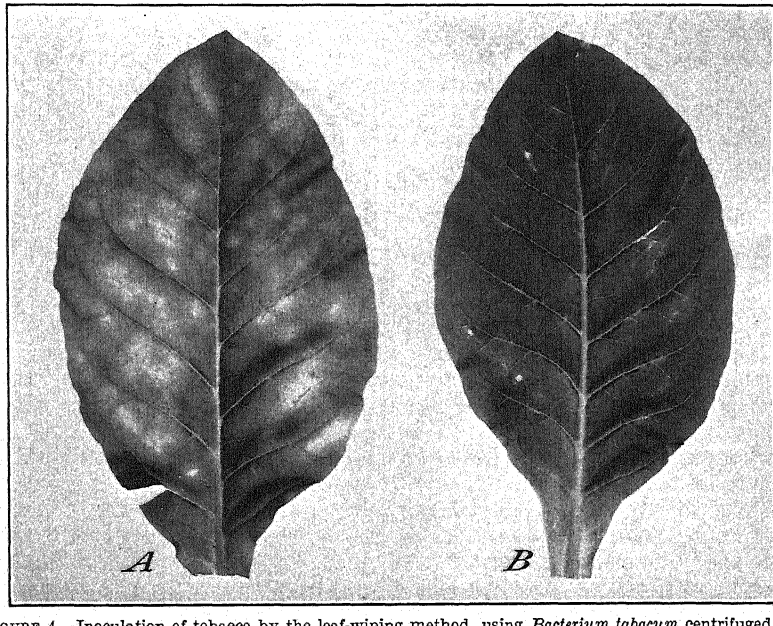


FIGURE 4.—Inoculation of tobacco by the leaf-wiping method, using *Bacterium tabacum* centrifuged free from toxin (*A*) and *Bact. angulatum* (*B*). The infective power of the former in the absence of water soaking is illustrated by the chlorotic areas resulting from numerous young infections. Spots on *B* are due to mechanical injury from wiping.

are most convenient for the equipment used. Twenty to forty minutes of water pressure was often required to water-soak 50 percent or more of the leaf area. The upper leaves usually water-soak more quickly than the older basal leaves, and the intercellular spaces nearest the midrib commonly fill first. However, the soaking is by no means uniform in the tobacco leaves, and the result is usually a wide variety of angles and patterns of varying sizes scattered over the leaf surface, which when allowed to proceed to certain degrees often bears a marked resemblance to the shape and distribution of angular leaf spot disease as it often occurs under field conditions (fig. 5).

If such water-soaked plants are taken out of the apparatus without inoculation and the roots or cut end of the stems placed in water at atmospheric pressure, transpiration at the lower humidities will rapidly remove the excess water in the leaves, but in a saturated atmosphere the water-soaked condition may remain up to 48 hours or more. The uninoculated recovered plants, even though kept under water pressure for 12 hours, show no sign of any physical internal or external injury to the tissues. The freedom from injury may be convincingly demonstrated by spraying water-soaked leaves of an F_1 hybrid (*Nicotiana tabacum* × *N. glutinosa*) with the virus of ordinary tobacco mosaic. If lesions of microscopic size are present, this host will develop marked necrotic lesions of virus infection. This absence of injury to the leaf surface by the internal water-pressure method has some advantages over the externally applied sprays from the point of view of illustrating the basic facts of infection.

The plants were sprayed once or twice with a DeVilbiss atomizer while under pressure, then set into milk bottles or Erlenmeyer flasks in water sufficient to cover the root system or stem end and placed in a saturated humidity chamber at about 25° C. Exposure in this chamber for as short a duration as 3 hours was sufficient to yield subsequent infection with some organisms on the tomato, but ordinarily the plants were left in the chamber until they showed signs of infection, which incubation usually required from 24 to 72 hours.

INFECTION WITH BACTERIUM ANGULATUM IN WATER-SOAKED TISSUES

When tobacco leaves water-soaked by internal pressure are inoculated with *Bacterium angulatum* over the entire surface, the necrotic lesions resulting often correspond closely to the water-soaked areas, and are usually of the irregular and angular type, resembling natural field infection as it often occurs (fig. 6). The blackfire lesions on leaves that are water-soaked by spraying the leaf surface (externally applied water pressure) more rarely show the sharply angular lesions, indicating the delimiting effect of the leaf veins on the lesion. No significance can as yet, however, be attached to this observation. Infections secured by the internal water-pressure method demonstrate in a convincing manner that the organisms enter through the stomata, in the absence of such cuticular wounding as may result from external sprays or storms. Clayton (*3*) suggests that the bacteria are shot directly into the stomata by the force of sprays or rain, and this is borne out in part by the fact that the careful dropping of water suspensions of the organism on the water-soaked area of the leaf is not as likely to yield infection as is light spraying.

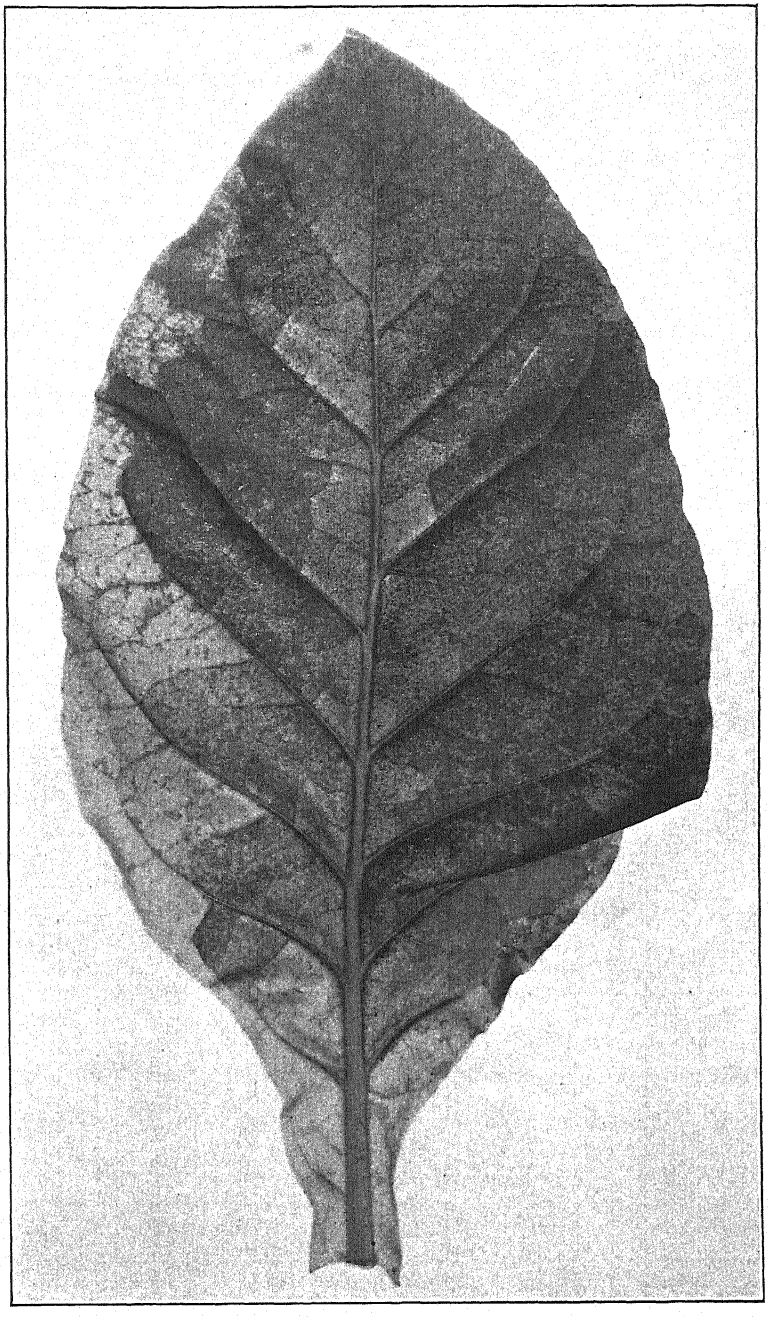


FIGURE 5.—A tobacco leaf from a plant water-soaked by the water-pressure method. The angular and speckled character of the lighter colored water-soaked areas is shown.

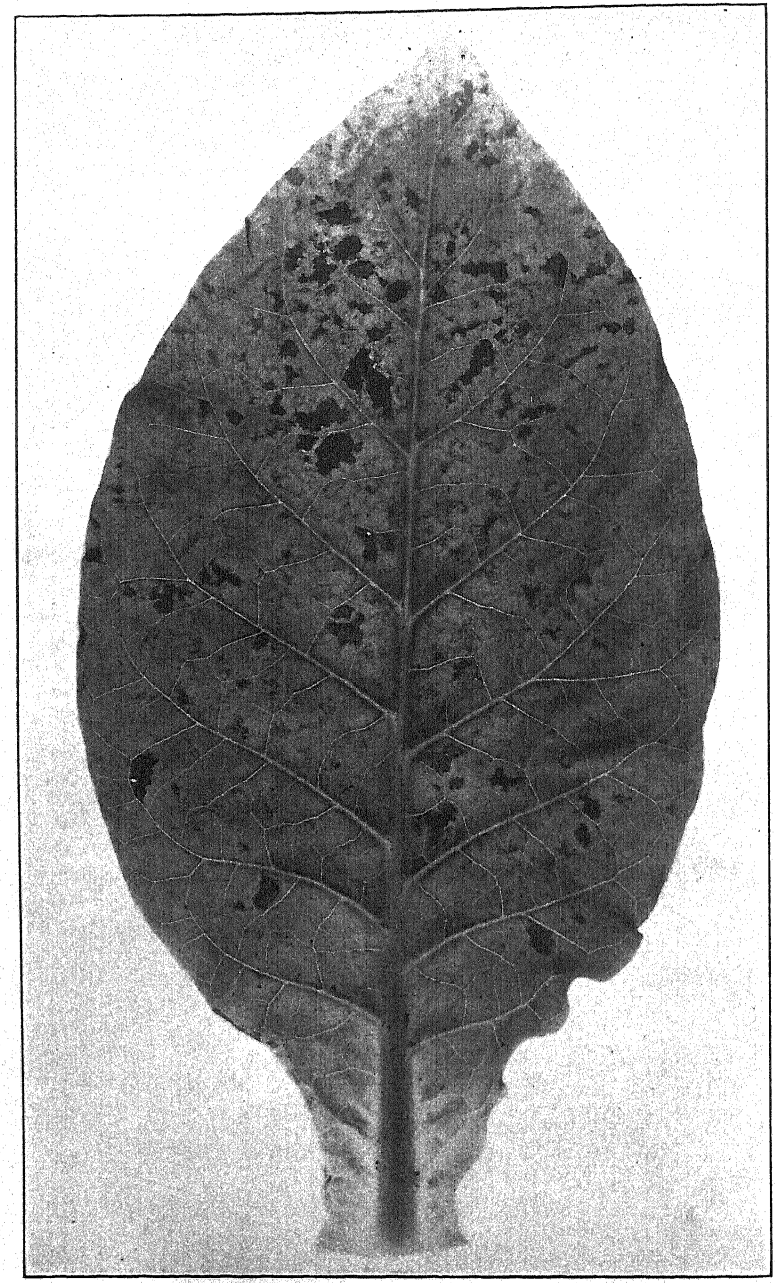


FIGURE 6.—A leaf of tobacco sprayed with *Bacterium angulatum* following water soaking by the water pressure method. The necrotic spots correspond roughly to the water-soaked areas and resemble the "epidemic" type of blackfire.

Bacterium angulatum, finding the water-filled stomatal chambers and intercellular spaces suitable for growth, multiplies rapidly and soon causes the cells to collapse, thus forming large necrotic areas (fig. 7). This action of the bacteria results in the epidemic or field

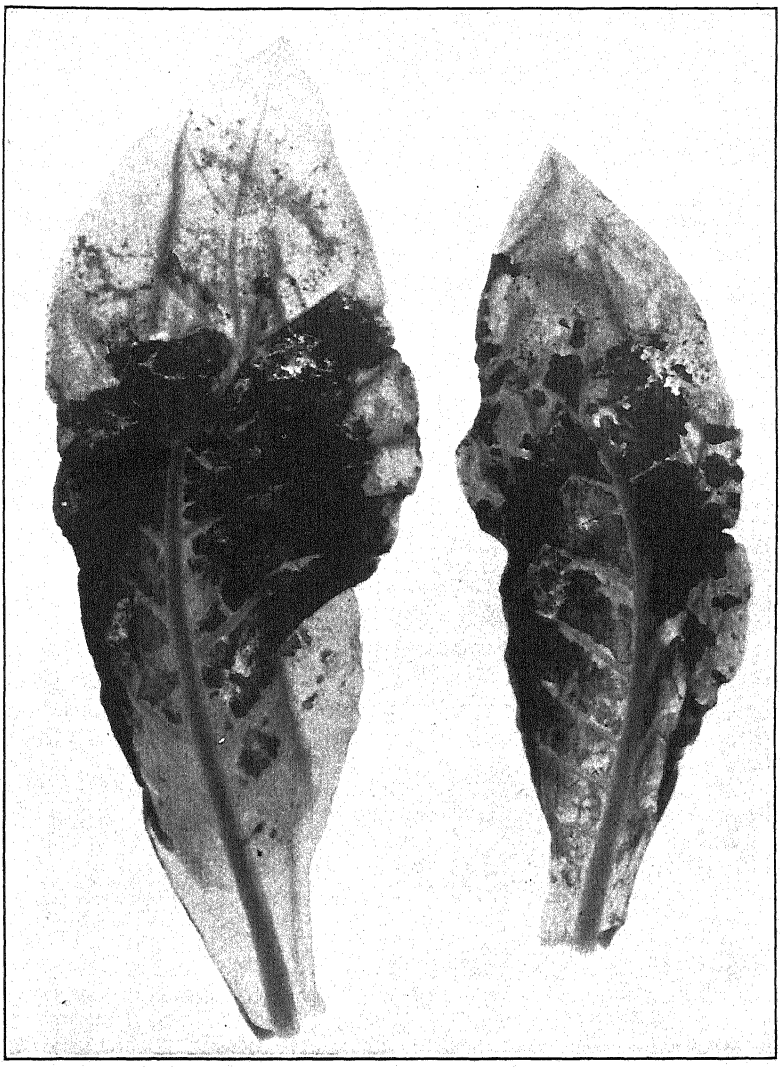


FIGURE 7.—Heavy water soaking of tobacco followed by spraying with *Bacterium angulatum* and continued duration of high humidity results in almost complete collapse of the leaves.

type of symptom as contrasted with the small or incipient type of lesion secured from inoculations without prior water soaking. The incipient lesions may, however, also arise from the pathogen's gaining a foothold in a water-soaked area of very small size. However, as Clayton (*3*) points out, for such lesions to develop, the water soaking

must be of sufficient duration to permit the organism to gain a foothold. The writer has found considerable variation in the time required for infection to occur in different host species as well as in individual plants of the same variety.

The greatly increased susceptibility of tobacco to *Bacterium angulatum* by the water-soaking method led the writer to try this organism on other hosts similarly treated. The results were most striking and unusual in many respects. The tomato plant, for example, proved much more susceptible than tobacco to the blackfire organism, and the leaves often collapsed completely in 24 to 48 hours, along with parts of the leaf petioles and younger portions of the stem (figs. 8, 9). Other



FIGURE 8.—*Bacterium angulatum* on two water-soaked tomatoes (*A*, *B*); control plant (*C*) water-soaked but not inoculated.

solanaceous plants tried, such as potato, eggplant, and datura, were not so markedly susceptible. On the other hand, when still other plants selected at random were used, it became clear that infection with *Bact. angulatum* by this method was not limited to genera or families but spread into a wide variety of unrelated families (figs. 10, 11).

The following species in particular were strikingly infected, some almost as severely as the tomato: Rose (*Rosa* sp.), poinsettia (*Euphorbia pulcherrima* Willd.), locust (*Robinia pseudo-acacia* L.), golden flax (*Linum flavum*), honeysuckle (*Lonicera morrowi* Gray), apple (*Malus sylvestris* Mill.), garden pea (*Pisum sativum* L.), marigold (*Tagetes patula* L.), geranium (*Geranium* sp.), hemp (*Cannabis sativa* L.), bean (*Phaseolus vulgaris* L.), ragweed (*Amaranthus retroflexus* L.), English ivy (*Hedera* sp.), alfalfa (*Medicago sativa* L.), clover (*Trifolium pratense* L.). Species on which infection was not secured with *Bacterium angulatum* included barley (*Hordeum vulgare* L.), corn (*Zea mays* L.), cabbage (*Brassica oleracea* L.), stock (*Matthiola incana* R. Br.), buckwheat (*Fagopyrum esculentum* Moench), snapdragon (*Antirrhinum majus* L.), cactus (*Zygocactus truncatus*), lemon (*Citrus* sp.). Over one-half of the species selected at random from the garden and greenhouse were readily infected with *Bact. angulatum* when inoculations were made to water-soaked tissue. Inoculations

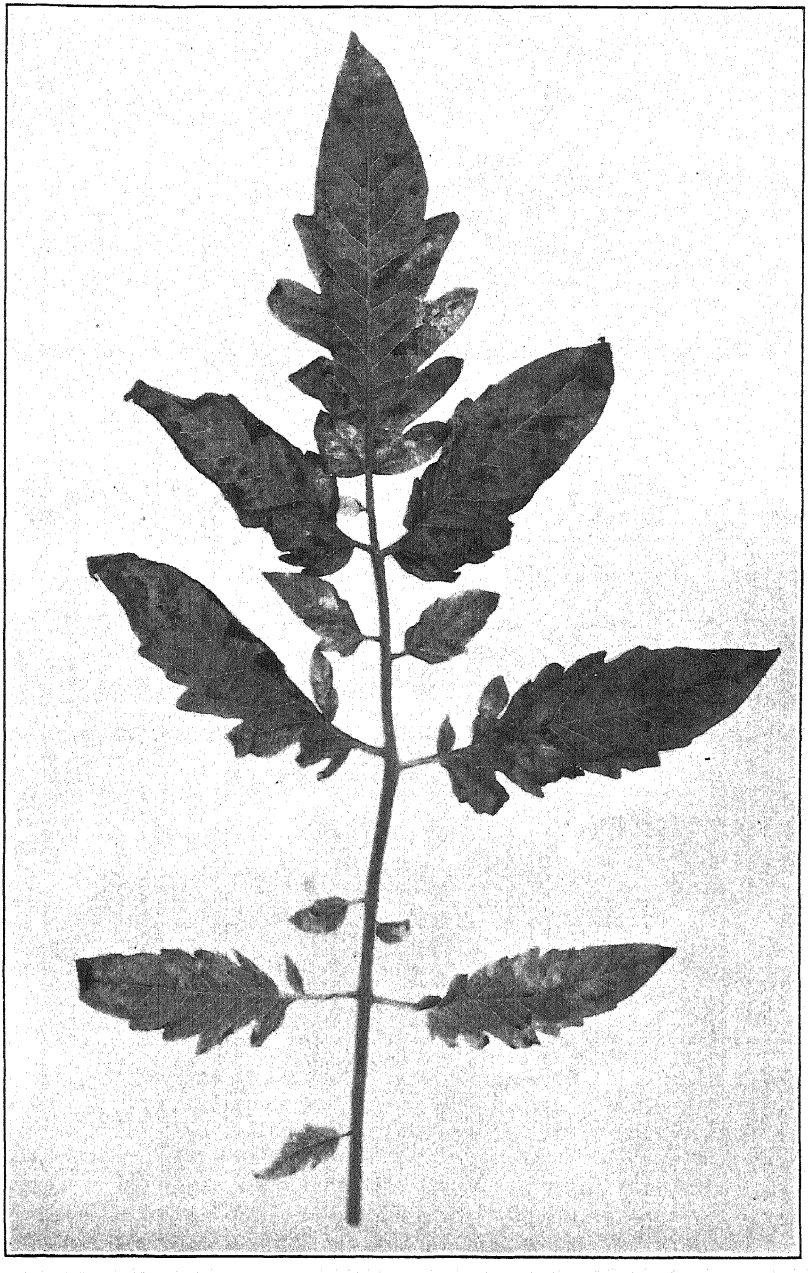


FIGURE 9.—Typical lesions of *Bacterium angulatum* on older water-soaked tomato leaf, which has not wholly collapsed.

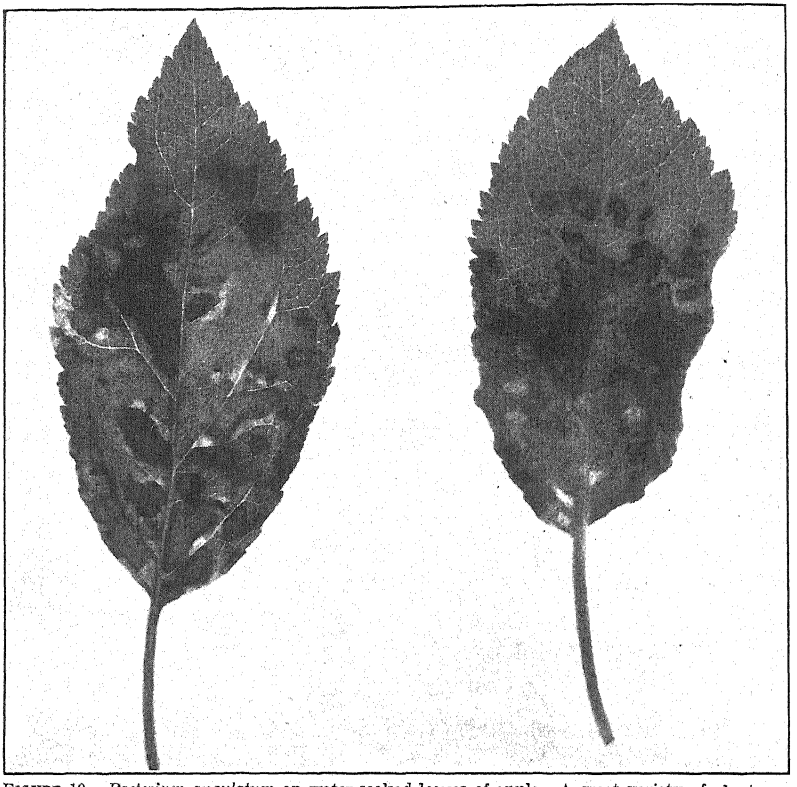


FIGURE 10.—*Bacterium angulatum* on water-soaked leaves of apple. A great variety of plants are equally susceptible after water soaking.

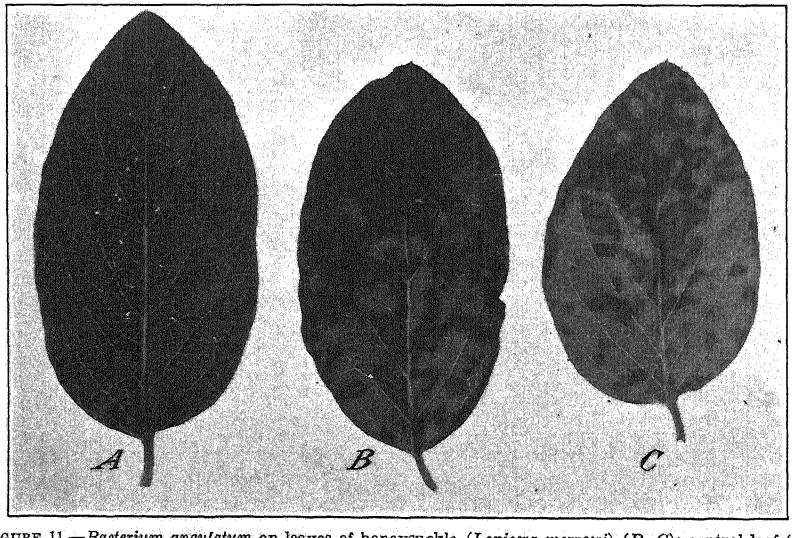


FIGURE 11.—*Bacterium angulatum* on leaves of honeysuckle (*Lonicera morrowi*) (*B*, *C*); control leaf (*A*) water-soaked but not inoculated.

on leaves of these species when not water-soaked yielded no symptoms in this series of trials.

INFECTION WITH BACTERIUM TABACUM IN WATER-SOAKED TISSUES

As is well known, the artificial inoculation of tobacco with *Bacterium tabacum* on tissues that are not water-soaked yields results which are distinct from those secured with *Bact. angulatum*. Under practically all expressions of the two diseases resulting from natural field infection, it is also possible to recognize wildfire because at least some halos are invariably present, and the angular and frequently black lesions of blackfire are characteristic. However, symptoms on tobacco from the two organisms on tissues artificially water-soaked by the internal pressure method are much less distinct and it becomes more difficult and sometimes impossible to separate them on the basis of symptoms.

When tomatoes water-soaked by the internal-pressure method are inoculated with *Bacterium tabacum*, the symptoms are identical with those secured with *Bact. angulatum*. The leaf tissue collapses, turns black, and no typical halos are produced, although should the leaf tissue fail to collapse, some general yellowing of the water-soaked area may be visible. Presumably the cells are killed before the toxin has sufficient time to form and act on the surrounding tissue, and the host or conditions are not sufficiently favorable to support the continued development of the organism and its toxin at the margins of the infected area. The advantage, if any, that the toxin furnishes to the parasitism of *Bact. tabacum* appears to be entirely absent in water-soaked tissues. The only difference that the writer has noted between the two organisms on tomato is the greater virulence of *Bact. tabacum* as indicated by the shorter duration of the water soaking required for the necrotic action to take place. Attenuated (1) strains of wildfire showing no toxin production are quite as virulent as are the normal strains.

Inoculations with *Bacterium tabacum* were made on water-soaked leaves of the same species used in the *Bact. angulatum* infection trials described above. Results almost identical in symptom expression with those found in the *Bact. angulatum* tests were secured in all instances, suggesting again the great similarity and close relationship of these two organisms.

INFECTION WITH OTHER ORGANISMS IN WATER-SOAKED TISSUES

The results with the blackfire and wildfire bacteria on water-soaked tissues can hardly be subject to sound interpretation without giving some consideration to the behavior of other plant parasites, or even saprophytes, under similar circumstances. The field of investigation at once becomes too extensive to be adequately surveyed in a preliminary paper, but a limited number of trials has shown that *Bacterium angulatum* and *Bact. tabacum* are not entirely unique in the above-described respects.

The results with some of the other organisms used have not been so uniformly consistent as those with *Bacterium tabacum* and *Bact. angulatum*, perhaps for the reason that the tomato is not a favorable host plant. Some probability of contamination of inoculated test plants by other organisms, where the same incubation chamber is used simultaneously for different organisms, has also been encoun-

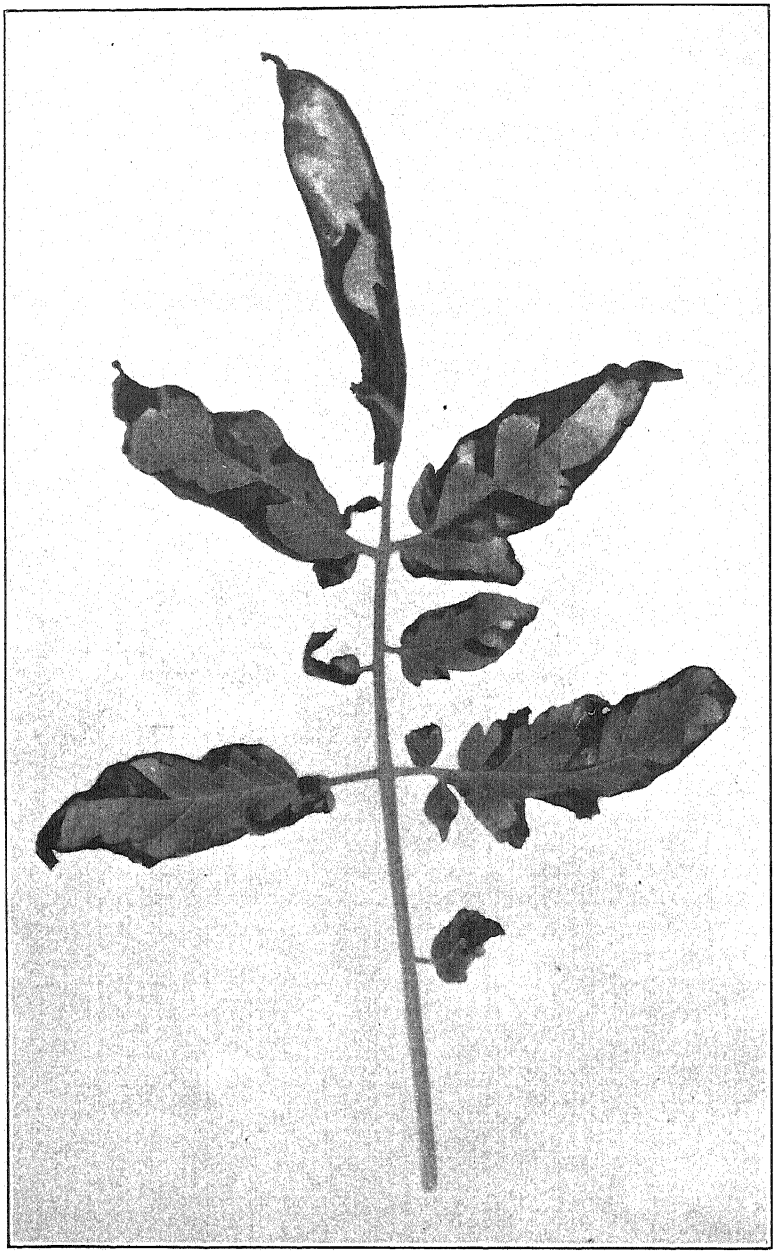


FIGURE 12.—A water-soaked tomato leaf inoculated with *Bacterium phaseoli*. The symptoms closely resemble those secured with *Bact. angulatum* and *Bact. tabacum*, but the progress of necrosis is much slower.

tered. Water-soaked uninoculated controls under these conditions have, however, invariably been free from symptoms. Fifteen other bacteria and two fungi have been tested, mostly in a limited way, especially on water-soaked tomatoes. Eight out of nine trials with *Bact. phaseoli* Smith on tomatoes gave definite and distinctive symptoms. The symptoms were somewhat less severe and slower in developing, but were much like those secured with *Bact. angulatum* or *Bact. tabacum* (fig. 12). Infection was also secured with *Bact. phaseoli* on potato, hemp, alfalfa, and marigold, but not on tobacco. *Bacillus carotovorus* Jones collapsed water-soaked tomatoes rapidly with symptoms readily distinguishable from those previously noted. *Bact. tumefaciens* Smith and Townsend, *Bact. punctulans* Bryan, and *Aplanobacter insidiosum* McC. yielded only small areas of blackened

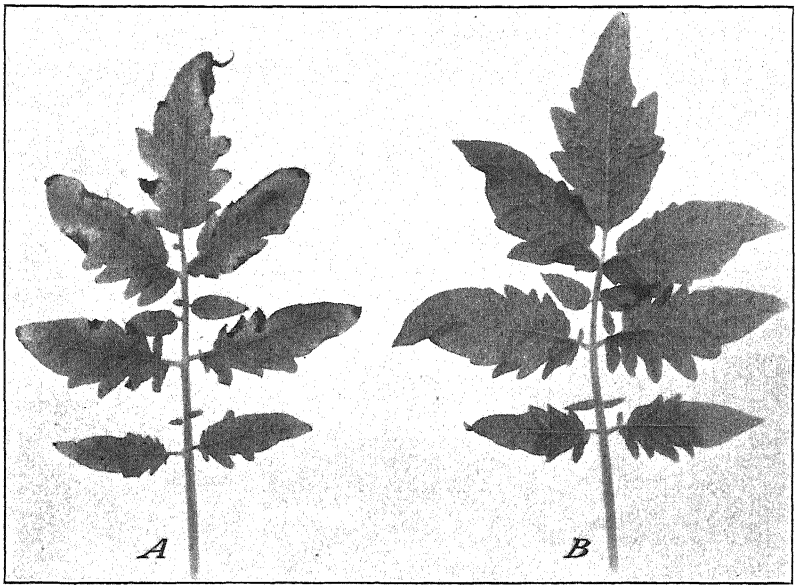


FIGURE 13.—*Macrosporium solani* on water-soaked tomato leaf (*A*) and on leaf without water soaking (*B*).

lesions of some uncertain character; *Bact. stewarti* Smith failed to give any signs of infection on tomato in three trials.

Infection experiments with fungus parasites on water-soaked tissues have been very limited thus far. Infection with *Macrosporium solani* Ell. and Mart. and *Septoria lycopersici* Speg. on tomato was favored by water soaking before inoculation (fig. 13). The latter fungus also evidently attacked potato readily under these conditions. Less conclusive but more surprising were the results sometimes secured with common bacterial organisms not belonging in the category of plant parasites. *Bacillus coli*[4] (Escherich) Migula, *Pseudomonas fluorescens* (Flügge) Migula, *B. radiobactar* Berij. and Van Deld., *B. ruber balticus* L. and N., *B. aerogenes* Kruse, and *Proteus vulgaris* Hauser produced mild necrotic areas on water-soaked tomatoes

[4] *B. coli* has previously been reported as attacking plant tissue. See references in Elliott, Charlotte. Manual of Bacterial plant parasites. Williams and Wilkins Co., 1930.

(fig. 14). Other organisms, including *B. prodigiosus* (Ehren.) Flügge, *B. subtilis* (Ehren.) Cohn, and *Staphylococcus aureus* Rosenbach, failed to produce any symptoms on tomato under similar conditions. In the above group of saprophytes particularly the writer is not prepared to state that true infection was definitely secured, and it should

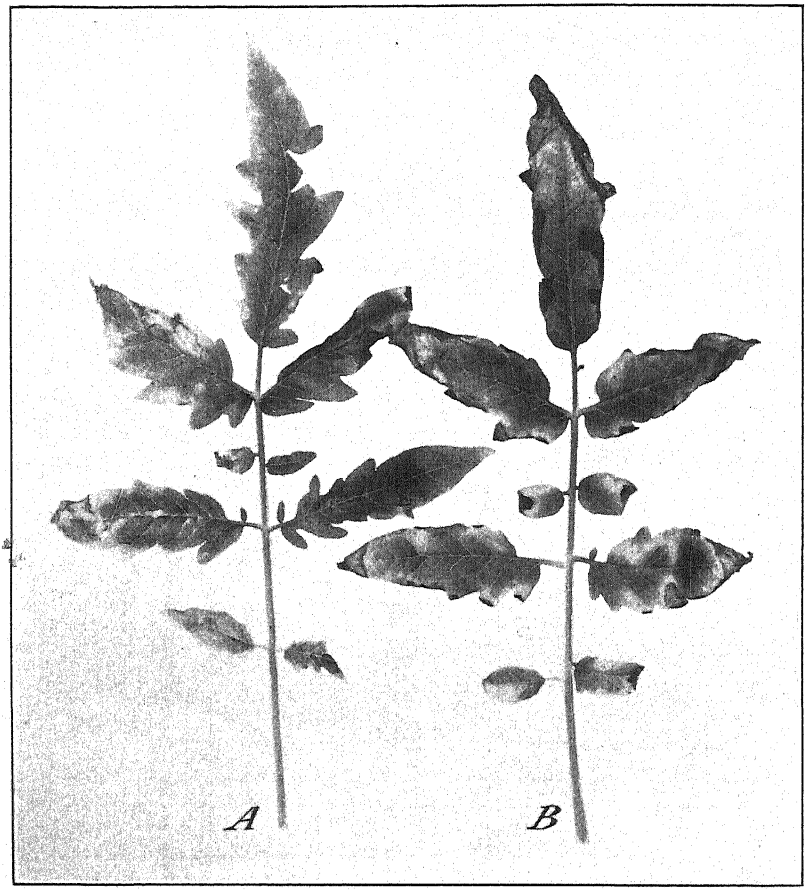


FIGURE 14.—Slow and poorly developed necrosis on water-soaked tomato leaves resulting from inoculation with *Bacillus ruber balticus* (*A*) and *B. aerogenes* (*B*).

be noted here that in no single case in these preliminary trials have Koch's postulates been carried to completion.

DISCUSSION OF RESULTS

It is apparent from the results presented that the infection secured with *Bacterium angulatum* on water-soaked tissues of normally immune species cannot be regarded as a form of saprophytism. The species evidently must be susceptible to attack by certain organisms, since many other species and organisms fail to respond in a similar manner to water soaking.

Since large numbers of organisms have been uniformly applied over the water-soaked areas, we know as yet comparatively little about the rate and distance of the development of the organism through the tissues. It is most likely that the progress from any one point of infection is comparatively localized, and it is obvious that the progress of the organism is limited by the area and the duration of the water soaking. The evidence taken altogether therefore goes to show that *Bacterium angulatum* is a comparatively weak parasite, and, as pointed out earlier by the writer (*5*), the significance of water soaking to infection is likely to be greatest with the less virulent parasites or the relatively more resistant hosts. It is perhaps of little consequence where the boundaries of the parasitism of organisms or the lines of immunity of the hosts are theoretically drawn. This subject resolves itself eventually into a matter of definition. It is significant, however, that these preliminary experiments suggest that a wide variety of normally immune host species may under some circumstances be temporary hosts in nature to *Bact. angulatum*, *Bact. tabacum*, and other organisms, thereby harboring them in such a manner that even the most thorough measures of sanitation and eradication of other known sources of infection may not suffice. Although there is reason to believe that the bacteria in question may persist in such host tissue in a dormant condition for a considerable time (*7*), the proof of this relation remains to be established. It is also possible that many of our so-called nonparasitic leaf spots in nature may be caused by organisms capable of infecting only water-soaked tissue. Isolates from such diseased tissue would naturally fail to give infection upon reinoculation by ordinary methods, and thus lead to erroneous conclusions, as suggested by Clayton (*3*) for the so-called nonparasitic blackfire described by Valleau (*10*).

It is believed that the experimental method of internal water soaking, as described, along with the external method of water soaking as used by Clayton (*3*), may have a wide application in furthering the present understanding of infection and progress of disease in plants. It is not unlikely that in nature a combination of both external and internal water pressure plays a role in predisposition to disease. It follows that more careful observations of water soaking as it occurs in nature, both as a result of beating rain (*3*) and root pressure (*5*), should be more generally made in relation to the epidemiology of plant diseases.

SUMMARY

The intercellular spaces of tobacco and other plant species were water-soaked by applying water pressure to the root system or cut stems, after which they were inoculated with *Bacterium angulatum* and other organisms.

Tobacco is normally very resistant to infection with *Bacterium angulatum*, but when tissues are water soaked, either by external or internal application of water pressure, and this condition is of sufficient duration, the tissues become very susceptible to the organism. No other set of environmental conditions for infection brought about the severe or "epidemic" type of this disease.

It is shown that this situation is not peculiar to tobacco or to *Bacterium angulatum* and *Bact. tabacum*. A wide variety of plant

species becomes equally susceptible to attack by these same organisms when the tissues are water-soaked. Excellent necrosis was secured on such plants as tomato, alfalfa, bean, pea, hemp, rose, apple, locust, flax, marigold, and poinsettia. These plants are normally immune to infection with these organisms. Other plant species tried were immune in the water-soaked condition.

Other plant parasites, such as *Bacterium phaseoli*, not normally capable of affecting tomato, for example, are capable of causing necrosis when inoculated into water-soaked tissues of this plant. A small amount of necrotic action was also secured on water-soaked tomatoes sprayed with such saprophytic species as *Bacillus coli*.

So far as can be determined, water soaking by the internal water-pressure method does not wound or injure the tissues, showing rather conclusively that the bacteria enter through the stomata, and that cuticular or epidermal wounding caused by rainstorms is not a fundamentally necessary condition for heavy field infection with *Bacterium angulatum* and *Bact. tabacum* as was previously supposed.

Modifications in the present conception of parasitism and immunity as regards definition of the terms are suggested by the results secured with water-soaked tissues. It is also likely that the results may have some practical bearing upon our present understanding of the sources of overwintering of certain plant parasites, and hence may modify the present theories of applying sanitary and eradication measures of disease control.

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EFFECT OF SPECIES OF HOST PLANT ON NITROGEN FIXATION IN MELILOTUS[1]

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INTRODUCTION

Early investigators of symbiotic nitrogen fixation believed that if the organisms invaded the plant and formed nodules there would be adequate fixation of elemental nitrogen, but later work showed this concept to be partly in error. Experiments in the controlled environment of the greenhouse and in the field yielded conclusive evidence that different strains of the bacteria benefit the host plant in varying degrees. By "strain" is meant any pure culture of the root nodule bacteria. In the course of many investigations there was observed a certain relation of bacteria and plant which is called host plant specificity. For example, an organism from vetch may be more beneficial to this plant than an organism from pea, although both strains will form nodules on the vetch. Here two genera are concerned, but in certain genera, notably in *Soja*, species and even varietal differences of response to invasion by a given strain of the proper species of bacteria have been reported. As the older work on strain variation and host plant specificity has been discussed by Fred et al. (*4*),[2] and by Allen and Baldwin (*1*), only a few of the more recent investigations will be reviewed.

Bjälfve (*2*) found differential varietal response among the vetches as to number and position of the nodules and quantity of nitrogen fixed. Virtanen (*8*) states that the most suitable strain of organism for one variety of peas is likewise the best for other varieties. He used only two strains of the pea organism; hence his observation may be true only for these two strains.

Examination of the literature reveals that most of the knowledge concerned with host-plant specificity has resulted from incidental observations in experiments made primarily to study strain variation among the bacteria. Few experiments are recorded in which the object has been to investigate specifically the possible role of the host in conditioning this variation in the organism. Because of the obvious importance and need of such studies, experiments have been conducted at this station for the past 3 years concerned with the specific problem of the influence of the host plant in the symbiosis. In this report are given the results of nitrogen-fixation tests on different species of *Melilotus* when inoculated with pure-culture strains of *Rhizobium meliloti*.

EXPERIMENTAL TECHNIQUE

Ten plants of different species of sweetclover were grown in half-gallon earthenware jars containing 2 kg of nitrogen-poor pit sand into which was inserted a watering tube. The methods for sterilization of seed and sand are given in previous publications (*5*, *10*). The plants were watered as needed with sterile, distilled water and once a week

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[2] Reference is made by number (italic) to Literature Cited, p. 629.

with sterile, nitrogen-free Crone's solution (*4*). In order to prevent contamination with foreign strains of the organism, the plants were protected by special chambers. These were made by covering a light wooden framework with a transparent rubber product called "Pliofilm." All jars inoculated with the same strain of bacteria were placed under the same chamber on low wooden trays mounted on rollers. The top of the chamber was hinged to facilitate the addition of water or nutrient solution. The efficacy of the method was demonstrated by the fact that uninoculated controls were thus kept free from nodules, although similar uncovered controls almost invariably became inoculated with stray organisms from the air. The trays bearing the jars were moved daily on the greenhouse bench so that variation arising from position in the greenhouse would be largely eliminated. When the growth of any of the plants was hindered by the light chamber, all were removed and the sand of each pot was covered with a thin layer of sterile cork. The cultures were usually 6 to 8 weeks under the light chambers and 2 to 3 weeks in the open air. At harvest the plants were washed free of sand and dry weight and total nitrogen determinations were made.

The species [3] of *Melilotus* used in these experiments were:

Melilotus alba (Hubam).—An annual-flowering white sweet clover (commercial seed).

Melilotus alba (32–19).—A tall late-flowering biennial from a single plant.

Melilotus suaveolens (F. P. I. 40937).—Redfield Yellow, a biennial developed at South Dakota.

Melilotus officinalis (Y–32–45).—Common yellow sweetclover. Biennial grown at this station for 20 years.

Melilotus officinalis (Y–33–33).—A fine-stemmed selection of yellow sweetclover.

Melilotus dentata (33–49).—Selection of nonbitter sweetclover from a single plant. Original seed was an annual from Peiping, China.

Melilotus dentata (Ac 91–92).—Selection from single plant grown in Wisconsin. Original seed collected in vicinity of Saratov, Union of Soviet Socialist Republics. Biennial.

Melilotus dentata (Ac 92–27).—Selection from single plant grown in Wisconsin. Original seed collected in West Siberia, Union of Soviet Socialist Republics. Biennial.

Melilotus dentata (Ac 96–2).—Selection from single plant grown in Wisconsin. Original seed from Busk, Poland.

The strains of *Rhizobium meliloti* used for artificial inoculation of the plants were:

No. 100—isolated in 1912 from Farmogerm alfalfa culture, a commercial inoculant.

No. 101—isolated in 1915 from United States Department of Agriculture alfalfa culture.

No. 105—isolated from nodule of alfalfa plant at Wisconsin, 1916.

No. 107—isolated from alfalfa culture of H. K. Mulford Co. in 1919.

No. 110—sweetclover cultures obtained from Illinois (Hansen).

No. 111—sweetclover culture from University of Illinois.

No. 113—isolated from sweetclover plant, University of Wisconsin, 1922.

No. 115—isolated from sweetclover plant, University of Wisconsin, 1922.

No. 128—isolated from nodule of bur-clover at Wisconsin in 1928.

No. 129—like 128 but separable by difference in electrophoretic property.

Since isolation the bacteria strains have been cultivated on artificial agar media (*4*), transferred monthly, and stored in an ice box.

EXPERIMENTAL DATA

Experiment 1 (Oct. 15, 1934, to Jan. 4, 1935) was a preliminary experiment in which 10 strains of the organism were used for inoculation of 3 species (2 varieties of 2 of the species) of *Melilotus*. The

[3] The authors express their appreciation to Prof. R. A. Brink, of the Department of Genetics, for the seed used in these experiments.

greenhouse was not provided with artificial illumination, and the plants were not protected with the light chambers. The experiment was started on October 15, 1934, and growth was satisfactory for the first 6 weeks. During the latter part of the experiment the development of the plants was very poor because of insufficient illumination. After 81 days harvest was made; the plants (triplicate jars) of each species (or variety) inoculated with the same same strain of bacteria were combined for analysis. The data are summarized in table 1.

TABLE 1.—*Growth and nitrogen fixation by different species and strains of Melilotus in association with various strains of Rhizobium Meliloti in experiments 1 to 3*

EXPERIMENT 1[1]

Rh. meliloti strain no.	*M. alba* (Hubam)		*M. alba* (32-19)		*M. officinalis* (32-45)		*M. officinalis* (33-33)		*M. dentata* (33-49)		*M. suaveolens*		Total nitrogen
	Dry weight	Total nitrogen	Dry weight	Total nitrogen	Dry weight	Total nitrogen	Dry weight	Total nitrogen	Dry weight	Total nitrogen	Dry weight	Total nitrogen	
	Grams	*Milligrams*	*Grams*	*Milligrams*	*Grams*	*Milligrams*	*Grams*	*Milligrams*	*Grams*	*Milligrams*	*Grams*	*Milligrams*	*Milligrams*
100	1.23	34.3	0.94	34.9	1.08	36.0	1.18	36.6	------	------	1.15	39.0	180.8
101	1.02	33.2	1.13	41.0	1.02	35.2	1.08	32.3	------	------	.47	15.8	157.5
105	.85	28.4	.95	36.9	.62	23.4	.83	28.2	------	------	.45	14.1	131.0
107	.98	29.5	.79	30.5	1.12	43.0	.91	32.8	------	------	.90	33.2	169.0
110	.89	29.1	.62	24.0	.58	22.8	.78	27.5	------	------	.72	26.4	129.8
111	.98	26.8	1.00	40.7	1.32	48.9	1.45	50.0	------	------	1.35	47.5	213.9
113	.96	29.8	.95	37.6	.95	31.2	.89	31.7	------	------	.82	29.3	159.6
115	.94	30.1	.77	30.6	1.20	45.8	1.13	40.8	------	------	.80	29.1	176.4
128	.98	29.8	.86	30.6	1.14	37.6	1.07	34.8	------	------	1.12	39.1	171.9
129	.99	27.4	.89	30.6	1.07	37.2	.83	24.4	------	------	.85	29.0	148.6
EXPERIMENT 2[2]													
100	4.35	81.8	2.92	80.5	3.68	88.9	------	------	2.41	54.5	3.20	83.6	389.3
105	3.34	75.0	3.06	74.6	3.15	78.0	------	------	.67	12.2	.82	21.8	261.6
110	3.07	70.5	2.93	75.6	3.14	80.0	------	------	3.23	68.0	4.06	92.5	386.6
115	3.27	80.3	2.43	65.0	3.38	79.9	------	------	3.15	73.0	3.54	90.6	388.8
128	2.88	71.0	2.42	65.4	2.76	80.0	------	------	1.97	44.5	3.76	97.5	358.4
EXPERIMENT 3[3]													
100	0.64	15.4	------	------	0.68	17.6	------	------	0.64	17.6	0.61	15.6	144.9
	.71	16.4	------	------	.92	22.0	------	------	.72	20.6	.68	19.7	
Average	------	15.9	------	------	------	19.8	------	------	------	19.1	------	17.7	
105	.48	13.4	------	------	.75	13.1	------	------	.19	3.2	.15	3.4	71.3
	.46	13.7	------	------	.70	12.6	------	------	.30	7.1	.22	4.8	
Average	------	13.6	------	------	------	12.8	------	------	------	5.2	------	4.1	
110	.98	18.5	------	------	.53	8.4	------	------	.84	21.6	.57	13.8	117.0
	.48	9.0	------	------	.87	11.3	------	------	.80	20.8	.60	13.6	
Average	------	13.8	------	------	------	9.8	------	------	------	21.2	------	13.7	
115	.79	14.7	------	------	.67	19.5	------	------	.80	17.9	1.20	13.7	146.6
	.68	15.5	------	------	.67	19.5	------	------	.93	25.4	.70	20.4	
Average	------	15.1	------	------	------	19.5	------	------	------	21.7	------	17.1	
128	.84	18.7	------	------	.35	9.4	------	------	.79	17.6	.97	23.4	123.6
	.83	13.7	------	------	.29	4.4	------	------	.41	11.8	.90	24.6	
Average	------	16.2	------	------	------	6.9	------	------	------	14.7	------	24.0	
Total	------	149.0	------	------	------	137.8	------	------	------	163.6	------	153.0	

[1] Dry weights per 10 plants; each datum based on analysis of 25 to 30 plants.
[2] Dry weights per 10 plants; each datum based on analysis of 30 plants.
[3] Dry weight per 10 plants; each datum based on analysis of 10 plants. Underlined values=mean of duplicates. Differences necessary for significance: Between means, 4.32 mg; between totals of strains, 17.3 mg. Differences between totals of species not significant (see table 4).

On the basis of the results obtained in the preliminary experiment five strains of the organism were shosen for further study. These were used for inoculation of plants of four species. The test was carried on from April 2 to June 23, 1935 in a greenhouse especially designed for control of environment in nitrogen-fixation studies (*10*), and the light chambers were introduced to prevent contamination. Growth and fixation of nitrogen were most satisfactory, as can be seen from the data in table 1 (experiment 2).

Experiment 3 (Sept. 30 to Dec. 15, 1935) was a replicate of experiment 2 except that only one variety of *Melilotus alba* was included. The work was done in rainy, cloudy weather, and in spite of artificial illumination the growth of the plants and the fixation of nitrogen were poor. However, this poor development was not entirely undesirable since the experiment was made in order to determine whether season and consequently development of plants affected the relationships between the host and invading bacteria.[4] The data are given in table 11. The data in the total columns are discussed in connection with the statistical analysis.

The data of experiment 1 show two types of variation in growth and fixation of nitrogen by *Melilotus* when inoculated with different strains of *Rhizobium meliloti*. With a given species of the host plant the benefit derived, as measured by the quantity of nitrogen fixed, varied with the strain of bacteria used. For example, with *M. officinalis*, strains 105 and 110 were definitely poor; strains 107, 111, and 115 were superior; the remainder were intermediate. With *M. suaveolens* strains 101 and 105 were poor and 100, 111, and 128 were distinctly good. This type of variation is the usual one noted by numerous investigators (*1*, *4*).

There are, however, also differences in nitrogen fixation of the several species of the host plant when inoculated with certain strains of the organism. For example, *Melilotus suaveolens* fixed less nitrogen in association with *Rhizobium meliloti* 101 and *Rh. meliloti* 105 than did any of the other species. These differences did not arise from variation in the growth habits of the various species of plant since with certain strains of bacteria (e. g., strains 100 and 110) no significant difference in the quantity of nitrogen fixed was found among the several species.

Confirmation of the existence of species variation with respect to fixation of nitrogen in *Melilotus* is obtained from the results of experiment 2 (table 1). This experiment was made under conditions ideal for fixation of nitrogen. The results show that with *M. alba* and *M. officinalis*, all the strains employed were apparently of equal benefit, but with *M. dentata* fixation was only fair with strain 128 and distinctly poor with strain 105. As noted in experiment 1, fixation by *M. suaveolens* in association with strain 105 was likewise very much less than with the other strains.

It is of interest to compare the results of experiment 2 with those of experiment 3 (table 1), made under environmental conditions that restricted fixation of nitrogen. In experiment 3 the analysis of the duplicate cultures was kept separate since, in contrast to the first two experiments, certain of the strains gave erratic reponses, a result that

[4] REID, J. J. THE INFECTIVE ABILITY OF RHIZOBIA OF THE SOYBEAN, COWPEA, AND LUPIN CROSS-INOCULATION GROUPS. Unpublished thesis, Ph. D., Univ. Wis. 1936.

has been observed by other investigators. Thus Wilson, Hopkins, and Fred (*11*) report that alfalfa plants inoculated with *Rhizobium meliloti* 101 and grown under cotton plugs (which also restricts uptake of elemental nitrogen) were benefited by the presence of this strain of the organism in only 7 of the 20 replicate cultures. Löhnis (*6*) reports a similar experience with crimson clover grown in open sand cultures when inoculated with *Rh. trifolii* 205. In general, the results of experiment 3 confirm those of the previous experiments in that both strain variation among the organisms and species variation among the host plants with regard to nitrogen fixation are apparent.

Further investigations of species variation in *Melilotus* were undertaken with those species that had exhibited the widest variation in the first three experiments. No more seed of *M. dentata* (33–49) was to be had, but seeds of three other strains of this species became available. These were tested with *M. alba* (32–19) and *M. suaveolens* in a preliminary experiment with *Rhizobium meliloti* strains 100, 105, and 128 as the source of inoculum. The average nitrogen fixed per 10 plants is shown in table 2.

TABLE 2.—*Nitrogen fixation by different species and strains of Melilotus in association with 3 different strains of Rhizobium meliloti in a preliminary experiment* [1]

Species or strain	*Rh. meliloti* 100	*Rh. meliloti* 105	*Rh. melilot* 128
	Milligrams	*Milligrams*	*Milligrams*
M. alba (32-19)	186.3	111.0	147.5
M. suaveolens	121.0	11.0	126.0
M. dentata (91-12)	149.0	95.0	131.0
M. dentata (92-27)	131.0	35.8	142.4
M. dentata (96-2)	111.5	142.0	138.2

[1] Per 10 plants.

The results of this experiment were very similar to those already discussed, with one exception. Of the three strains of the biennial *Melilotus dentata* used, only one, *M. dentata* (92–27), responded to inoculation with *Rhizobium meliloti* 105 as did the annual, *M. dentata* (33–49). Fixation of nitrogen was fair to good with *M. dentata* (91–12) and was good with *M. dentata* (96–2). This result indicates that the importance of the host plant in nitrogen fixation by *Melilotus* is not confined to species differences but may likewise include varietal (or strain) differences.

In order to examine this problem further two additional experiments (nos. 4 and 5) were made. The results of these experiments are given in table 3. Only the nitrogen fixation data are included since these are the essential ones and the dry-weight figures were consistent with these.

The results of the two experiments confirm in every way those of the previous ones; viz, the existence of three types of response in the association of *Rhizobium meliloti* and *Melilotus*.

(1) With *Rh. meliloti* 100 and 110 fixation of nitrogen by all the species of host plants was good to excellent with little evidence of consistent significant differences among the several species. The results were consistent in that agreement between duplicate samples was satisfactory.

(2) With *Rh. meliloti* 105 evidences of the effect of the host plant were quite pronounced. Fixation of nitrogen in association with *M. alba* (32–19) and *M. dentata* (91–12) was fair to good, but fixation with both *M. suaveolens* and *M. dentata* (92–27) was poor. The response was quite definite and the agreement between duplicates was satisfactory.

In experiment 4 (Oct. 23, 1936, to Feb. 2, 1937) three species of *Melilotus* were inoculated with the same five strains of organisms which were used in the earlier experiments. *M. Alba* (32–19) was used as representative of the *M. alba* species since it had shown more indication of variable behavior with the organisms used than had the Hubam variety. Likewise, the two strains of *M. dentata* which had shown the greatest contrast in the preliminary experiment were selected for further study. The experiment was made during the winter months, and in spite of the use of supplemental lighting, fixation of nitrogen was only fair even with the best of the associations.

In experiment 5 (Feb. 16 to May 21, 1937) only *Rhizobium meliloti* 105, 115, and 128 were used, as the results in the previous experiments indicated that there existed little, if any, consistent differences in the response of the species of host plant under investigation when inoculated with strains 100 and 110. The experiment was started in the late winter and the initial growth of the plants was slow. Later in the spring months growth was excellent and fixation was the greatest obtained in any of the experiments, due in part to the longer period of growth allowed the plants.

TABLE 3.—*Nitrogen fixation by different species and strains of melilotus in association with various strains of rhizobium meliloti in experiments 4 and 5* [1]

Rh. meliloti strain no.	*M. alba* (32–19)		*M. suaveolens*		*M. dentata* (91–12)		*M. dentata* (92–27)		Total	
	Experiment 4	Experiment 5	Experiment 4	Experiment 5	Experiment 4	Experiment 5	Experiment 4	Experiment 5	Experiment 4	Experiment 5
	Milligrams	*Milligrams*	*Milligrams*	*Milligrams*	*Milligrams*	*Milligrams*	*Milligrams*	*Milligrams*	*Milligrams*	*Milligrams*
100	20.9 23.3	-------	24.1 22.0	-------	20.5 23.5	-------	22.1 28.1	-------	181.5	-------
Average [2]	22.1	-------	23.1	-------	22.0	-------	25.1	-------	-------	-------
105	15.4 17.4	163.3 213.0	4.1 3.2	12.9 10.3	15.5 18.3	240.5 268.1	5.8 7.4	55.0 40.0	87.1	1,003.1
Average [2]	16.4	188.2	3.7	11.6	16.9	254.3	6.6	47.5	-------	-------
110	21.3 20.2	-------	23.3 25.6	-------	19.5 20.5	-------	30.2 32.0	-------	192.6	-------
Average [2]	20.8	-------	24.5	-------	20.0	-------	31.1	-------	-------	-------
115	23.1 20.9	144.0 128.6	26.9 30.7	250.2 284.0	27.4 31.8	224.2 230.6	10.2 11.0	117.5 215.0	182.0	-------
Average [2]	22.0	136.3	28.8	267.1	29.6	227.4	10.6	166.3	-------	1,594.1
128	28.2 27.5	238.0 294.1	18.1 27.3	248.1 170.1	22.6 28.6	193.5 209.5	14.1 24.1	227.5 238.6	190.5	-------
Average [2]	27.9	266.1	22.7	209.1	25.6	201.5	19.1	233.1	-------	1,819.4
Total	218.2	1,181.0	205.3	975.6	228.2	1,366.4	185.0	893.6	-------	-------

[1] All values in milligrams per 10 plants. Differences necessary for significance: Experiment 4, between means, 4.33 mg.; between totals of strains, 17.3 mg.; between totals of species, 19.3 mg. Experiment 5 between means, 48.7 mg.; between totals of strains, 194.8 mg.; between totals of species, 168.7 mg.

[2] Mean of duplicates.

(3) In association with strains 115 and 128 certain species exhibited another type of variation with respect to fixation of nitrogen. All the species fixed appreciable quantities of nitrogen with these two strains, but in association with *M. suaveolens* and *M. dentata* (92–27) the response was inclined to be erratic, with the result that agreement between the duplicates was poor. This type of response was discussed in connection with experiment 3, and its possible significance will be taken up later. The time element in the development of the differences between duplicates in experiment 5 is of interest. In the early stages of growth both cultures of a given treatment developed slowly with little sign of difference between them. As the days became longer and brighter one of the cultures would respond to the environmental conditions more favorable for the fixation of nitrogen sooner than its duplicate, with the result that differences in fixation of nitrogen soon became apparent between the two. Later both cultures would actively fix nitrogen, but the one in which the onset of fixation was delayed could not overcome the initial advantage of the other so that the agreement between the duplicates was poor. It should be observed that this difference between duplicate cultures in the onset of the fixation process appeared to be definitely associated with only certain species when inoculated with certain strains of the organism.

The conclusions reached by inspection of the data were confirmed by subjecting them to an analysis of variance (*3*). Such an analysis allows separation of the different sources of variation in the results and enables the establishment of the significance of variation arising from differential response of species of host plant with strain of bacteria. Analyses of variance (table 4) were made on the data from experiments 3, 4, and 5 as in these experiments the duplicates were not combined. The values necessary for significant (19:1 odds) differences between the means of the duplicate samples of each experiment (calculated from the error of the experiment) as well as for total nitrogen fixed by the various strains of bacteria or by the various species of host plant are indicated in the footnotes to tables 1 and 3.

TABLE 4.—*Summary of analyses of variance on experiments 3, 4, and 5*

Variance due to—	Experiment 3			Experiment 4			Experiment 5		
	Degrees of freedom	Variance	F [1]	Degrees of freedom	Variance	F [1]	Degrees of freedom	Variance	F [1]
Strains	4	115.3	13.31++	4	253.8	29.6++	2	21,634.2	21.6++
Species	3	10.86	1.26−	3	33.76	3.94+	3	6,301.6	6.3++
Species × strain	12	51.0	5.92++	12	72.2	8.42++	6	13,946.1	13.9++
Error	20	8.63		20	8.58		12	1,000.3	

[1] If F exceeds value for 5-percent point (indicated by +), the odds are at least 19:1 that observed differences do not arise from chance (experimental error). Similarly, if F exceeds 1-percent point (indicated by ++) odds are 99:1. Minus indicates differences could have arisen from chance.

The source of variation of chief interest is the interaction of species and strain (species × strain), for if this is significant the fixation of nitrogen by a given strain of the organism in association with the host plant is not independent of the species of the host. The analyses show that, in spite of considerable experimental error introduced by

reason of the difference in duplicate samples that has been already discussed, the variation due to interaction of strain and species is highly significant in each of the experiments.

Likewise, in the three experiments for which analyses of variance are reported (table 4) there are significant differences due to strain. Consideration of table 1 (experiment 3) and table 3 indicates that this difference arises primarily because strain 105 is distinctly inferior to the others when the results on all the species are combined. It is to be emphasized that this does not mean that strain 105 is inferior on all the species when considered individually as with certain of them; e. g., on *Melilotus alba* (32–19) and *M. dentata* (91–12) strain 105 usually fixed as much nitrogen as did the other strains. Because of the consistently poor fixation by strain 105 in association with *M. suaveolens* and *M. dentata* (33–49) and (92–27), however, the total fixed by strain 105 is significantly lower than the totals fixed by the other strains used.

Similarly, the significance of the species variation arises from distinctly lower totals for *Melilotus suaveolens* and *M. dentata* (92–27), lower totals which originate primarily from the poor fixation when inoculated with strain 105. With other strains of the bacteria, e. g., strain 100, the quantity of nitrogen fixed by either of these two species is not significantly different from the nitrogen fixed by the other species of host plant.

DISCUSSION

From the results of the foregoing experiments it is concluded that the interaction of bacteria and host plant in symbiotic nitrogen fixation by *Melilotus* may be separated into three distinct types of response with respect to nitrogen fixation.

(1) The association of all the species tested with certain of the organisms is consistently of equal effectiveness. The best examples of this type are *Rhizobium meliloti* 100 and 110 in association with the species under investigation. Probably other strains of the bacteria tested only in experiment 1 would also fall in this class with these particular species of host plant. It should be noted, however, that other species (or even varieties) of *Melilotus* may be found in which the association with strains 100 or 110 would be ineffective.

(2) A second type of association is that in which the species of host determines whether or not the strain of bacteria is effective. For example, *Rh. meliloti* 105 is effective in association with *M. alba*, *M. officinalis*, and *M. dentata* (91–12) but rather ineffective with *M. dentata* (33–49) and (92–27) and with *M. suaveolens*. This type is likewise consistent; i. e., the response of a given species to inoculation with the strain is relatively independent of the environmental conditions under which the experiment is made.

(3) In the third type of association observed in these experiments certain of the host plants and strains exhibited a variable response which appeared to be related to the environmental conditions under which the experiment was made, especially length of day and intensity of light. The most consistent examples of this type was the association of *Rh. meliloti* 115 and 128 with *M. suaveolens*, *M. dentata* (33–49) and (92–27). It is of interest that these species of host plant are the ones which are ineffective in association with *Rh. meliloti* 105.

In many cases the variation is apparent between replicates in the same experiment. The cause of this irregularity is even more obscure than the cause of the differences in effectiveness between individual strains, but it is doubtless a reflection of a fundamental change in the delicate equilibrium existing between host and invader which determines whether the relationship becomes helpful or harmful to the plant. In view of the importnce of the carbohydrate-nitrogen relationship of the host in influencing both effectiveness and ineffectiveness (*9*), it appears that this relationship may be concerned in the inconsistencies noted with certain strains and species. This suggestion receives support from the fact that irregularities are more frequently observed when the experiments are carried out under conditions which favor a narrow carbohydrate-nitrogen balance in the, plant and which consequently restrict fixation of elemental nitrogen at least during the early stage of development. It is further suggested that the inconsistencies in the quantity of nitrogen fixed through certain associations of host plant and bacteria may arise from differences in time of infection by the bacteria rather than irregularities in the effectiveness of the association. Unpublished results by Reid[5] have indicated the importance of the carbohydrate-nitrogen relation in determining whether or not a given strain of the bacteria will infect certain of the host plants of the soybean-cowpea cross-inoculation group. Under an unfavorable environment, invasion of individual plants by certain strains of the bacteria may occur at different periods, depending on what appears to be minor factors in the environment, but which bring about changes in the composition of the host plant sufficient to control the time of infection.

The implication of the findings for theoretical aspects of symbiotic nitrogen fixation is the emphasis which they place upon the influence of the host plant in the symbiosis. The assumption that the host plant passively supplies the source of energy and that the actual process of fixation is a function of the bacteria alone appears to be untenable in the light of these results. Research in the field has been largely concerned with efforts to determine the individual roles of the plant and organism, but such an approach may be unwise. Regarding the fixation of nitrogen as a function of the relationship between host and organism acting as a unit rather than as isolated components, might prove to be a more rational point of view for future research.

A corallary of this view of mutual interdependence of bacteria and plant is the necessity of change in the concept of what constitutes "strain variation"—a broader definition which would include the host plant might be advisable. With our present definitions *Rhizobium meliloti* 105 would be classed as "effective" with certain hosts but "ineffective" with others. But if the variation is considered as one involving the relationship between plant and bacteria rather than merely the strain of the organism (or the species of plant) a more unified conception is possible.

According to this concept the difference in strains of the organism is not so much a definite qualitative one capable of classification in terms as "effective" and "ineffective", but rather a quantitative difference in the manner in which the bacteria affect the relationship between themselves and the host. Moreover, this quantitative

[5] REID, J. J. See footnote 4.

difference is not necessarily constant but may vary in relation to the presence and activities of other factors which likewise affect the relationship. These factors would include the species of the host plant, the carbohydrate-nitrogen relation in the plant, and probably others; such as day length, temperature, and light intensity. Thus the irregularity in the fixation of nitrogen with some cultures, as *Rhizobium meliloti* 128 with *Melilotus dentata* (33–49) and *M. suaveolens*, *Rh. meliloti* 101 with *Medicago sativa* (Grimm) (*12*), and *Rh. trifolii* 205 with *Trifolium incarnatum* (*6*), constitute examples of alteration of the quantitative effect of a strain on the association through the predominance of some other factor—possibly the carbohydrate-nitrogen relation in the plant—which affects the relationship. The objective of future research is to define the as yet unknown factors, to determine their relative significance especially in combination with one another, and finally to devise means for their control.

SUMMARY AND CONCLUSIONS

Four species of sweetclover, *Melilotus alba*, *M. officinalis*, *M. suaveolens*, and *M. dentata*—were tested for ability to fix atmospheric nitrogen in association with different strains of *Rhizobium meliloti*. Six experiments were made during different seasons with resulting differences in extent and rate of nitrogen fixation by the plants.

Three types of response with respect to fixation of nitrogen were observed in these experiments:

(1) The association of certain of the species of host plants and strains of bacteria were consistently effective.

(2) The association of certain strains of bacteria was effective with one species of the host but ineffective with another. The reverse was also observed, i. e., certain species of the host plant were benefited through association with some strains of the organism but not with others.

(3) The association of certain species of plant and certain strains of the organism gave rise to erratic responses between experiments as well as within an experiment. There was some evidence that this type of response may have been influenced by the carbohydrate-nitrogen relationship in the plant.

These results stress the importance of the host plant in determining whether a given strain of bacteria is effective or ineffective in the association. It is proposed that the concept of strain variation in the bacteria may be only one aspect of a broader type of variation which involves the total relationship between organism and plant. The variation in this relationship with respect to nitrogen fixation may be affected by factors other than differences of strain of bacteria, as, for example, species of host plant or physiological balances within the plant. According to this view a given strain of the organism is not "good" or "poor" in an absolute sense, but only relative to the other factors which affect the relationship. Under certain conditions one factor, as, for example, strain of organism, may determine the effectiveness of the relationship, whereas under other conditions some other factor, such as species of host plant, may be the dominating element.

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THE NATURE AND INTERACTION OF GENES CONDITIONING REACTION TO RUST IN FLAX [1]

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INTRODUCTION

Flax rust, caused by *Melampsora lini* (Pers.) Lév. is widespread throughout the flax-growing regions of the world, and in certain years epiphytotics of serious proportions occur, causing severe losses to growers. The threat of rust epiphytotics in Minnesota has been sufficiently great to be a major factor in causing the removal from the recommended list of the two susceptible varieties Chippewa and Winona, and at the present time makes it imperative that new varieties of flax to be recommended for commercial production be highly resistant to, or immune from, rust.

The physiologic-form concept has served to emphasize the necessity for producing varieties resistant to all races of the pathogen prevalent in a locality. Consequently, the inheritance of reaction to a collection of rust is of primary importance in breeding for rust resistance in flax. Likewise, a knowledge of the degree of association between the reaction of hybrids to a collection of rust and to a single physiologic race commonly found in the region is of considerable importance both from a practical and from a theoretical viewpoint.

The purpose of this study was threefold: (1) To determine the nature of interaction of genes conditioning different types of rust reaction; (2) to determine the relationship and interaction of genes conditioning a similar reaction type in different varieties; and (3) to determine the relationship between the reaction of hybrids to a collection of rust and to a single physiologic race.

LITERATURE REVIEW

Studies of the inheritance of disease resistance in crop plants have been a fruitful field for the application of genetic principles to plant breeding, and the literature pertaining to such studies is voluminous. Likewise, the morphological characters of flax have been the subject of rather extensive genetical researches, particularly by Tammes. This literature has been reviewed in recent papers by Tammes (*9*, *10*)[3] and the writer (*7*). It is a singular fact, however, that relatively little work has been reported on the inheritance of reaction to rust in flax.

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[3] Reference is made by number (italic) to Literature Cited, p. 666.

In 1921, Henry (*5*) stated that several previous investigators had observed a differential rust reaction in different varieties of flax, some reporting immune varieties. At the same time, he reported that numerous varieties had been immune from rust in tests in Minnesota. Later, (*6*) he stated that Ottawa 770B, Argentine Selection, and Bombay had remained consistently immune when thoroughly tested with several collections of rust from the United States and Canada, and that all three had likewise been immune when inoculated with a collection of rust furnished by Tammes from her flax-breeding gardens at the University of Groningen, Groningen, Netherlands. In addition, Henry (*6*) stated that Hiratsuka tested Ottawa 770B and Argentine Selection in Japan with the same results.

Henry (*6*) studied the inheritance of immunity in crosses involving Argentine Selection, Ottawa 770B, and Bombay as the immune parents. The immunity of Ottawa 770B and Bombay was in each case dependent upon a single dominant factor. In Argentine Selection, apparently two dominant factors were present, either of which conditioned immunity. In crosses involving Ottawa 770B, rust reaction and flower color were inherited independently.

Recently, Flor (*3*) has reported the differentiation of 14 physiologic forms of *Melampsora lini* by the use of seven varieties of cultivated flax. Of the 165 varieties of flax inoculated with forms 1 to 5, only 13 gave indications of being rust differentials, the remaining 152 showing no specific response to the 5 forms of rust. Flor states that the lack of genetic purity of the varieties with regard to rust reaction was one of the striking features of his results.

MATERIAL AND METHODS

The following varieties of flax (*Linum usitatissimum* L.), used as parents in the crosses reported in this paper, are grouped according to their supposed rust reaction at the time this study was outlined:

Immune: Ottawa 770B, C. I. 355 [4]; Newland, C. I. 188; Minnesota Selection, C. I. 438; Long × E, C. I. 697; Pale Blue Verbena, C. I. 416–3; and Argentine Selection, C. I. 712.

Resistant: Light Mauve, C. I. 379–1; Pale Pink, C. I. 649; Bison, C. I. 389; Redwing, C. I. 320; and Ottawa 829c, C. I. 391.

Moderately susceptible: Bolley Golden, C. I. 644; Pale Blue, C. I. 423; and Abyssinian Yellow, C. I. 300.

Susceptible: Common Pink, C. I. 479.

The data on rust reaction, used in outlining this study, were made available to the author by C. C. Allison of the Division of Plant Pathology and Botany, University of Minnesota, and were from notes taken on the varieties grown at the Coon Creek Experimental field in 1932.

The parental material, wherever possible, was taken from rows descending from individual plants. However, such rows were not available of Newland, Long × E, C. I. 416–3, C. I. 712, Light Mauve, and Bolley Golden, and for these varieties it was necessary to plant bulk seed for making crosses. A total of 37 crosses was made. Varieties representing different types of rust reaction were intercrossed, and, in addition, varieties showing a similar type of reaction were crossed in all combinations. The crosses were made in the greenhouse during

[4] C. I. refers to accession number of the Division of Cereal Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture.

the winter of 1933–34, and the parents and F_1 plants were grown on peat soil at the Coon Creek experimental field in 1934. In addition, an F_2 generation of Ottawa 770B × Pale Blue, and F_2 and F_3 generations of Ottawa 770B × Redwing were grown in space-planted rows. Additional hybrid seed of each of the crosses was obtained in the flax-crossing plot in 1934.

F_2 generations of all crosses were grown in 1935 at University Farm, Minn., and on the Coon Creek peat. However, because of adverse climatic conditions, no data were obtained on rust reaction of the hybrid material during this summer.

In the greenhouse studies of rust reaction, conducted during the winter of 1935–36, F_1 and F_3 lines were used in all crosses where seed was available. In certain crosses where there were insufficient F_3 lines F_2 material was also used. Each F_3 line was planted in a single 4-inch pot at the rate of 20 seeds per line where sufficient seed was available. Likewise, the F_2 generations and the parental checks were planted at the rate of 20 seeds per 4-inch pot.

The collection of rust was obtained at University Farm from the variety Winona, previously inoculated with rust collected at Coon Creek. Physiologic form 4 was obtained from H. H. Flor, of the United States Department of Agriculture. Both the collection and the single form were increased in the greenhouse on Winona.

A temperature of about 70° F. was found to be suitable for growing the flax and for producing good rust infection. Two large incubators, each with a capacity of seventy-five 4-inch pots, were available and an incubation period of 48 hours was used. As a result, about 150 pots were planted every second day. The first planting was inoculated by the brushing method, with the collection of rust, 3 weeks after the date of planting. The plants were then 4 to 6 inches tall. Inoculations were continued every other day on the subsequent plantings until in November, when the low light intensity and duration retarded the growth of the flax. Since it seemed desirable to inoculate all crosses at nearly the same stage of growth, the period from planting to inoculation was gradually extended from 3 weeks to 4 weeks in December. As soon as seed of all the material had been planted for testing with the collection of rust, a duplicate planting was started for testing with the single physiologic race. The procedure followed with the material for inoculation with the single race was essentially the same as that for the collection.

In taking the notes, no standardized classes were available as is the case in seedling studies with the cereals, particularly wheat. Consequently, it was necessary to set up the classes as the notes were taken. Eleven classes were used, based upon extent and type of infection. These classes were as follows:

Class 0: Plants showing no macroscopic evidence of the presence of the pathogen (pl. 1, *A*).

Class 1: Plants with small flecks but no pustules (pl.1, *B*).

Class 2: Plants with flecks and very small pustules, the pustules surrounded by a narrow necrotic area or by chlorotic tissue (pl. 1, *C* and *D*).

Class 3: Plants with flecks, very small pustules, and occasional medium large pustules intermingled on the same leaves over the entire plant. Pustules usually surrounded by a necrotic area although occasional pustules were surrounded by a chlorotic area (pl. 1, *E*).

Class 4: Plants with few small pustules (less than four or five per leaf) surrounded by necrotic areas on older leaves. Leaves on the upper one-third of the plant with large susceptible pustules, surrounded by chlorotic area (pl. 1, F).

Class 5: Plants like class 4 except with more pustules per leaf (pl. 1, *G*).

Class 6: Plants with large pustules surrounded by chlorotic areas on all leaves. Usually two, three, or four pustules per leaf surface (pl. 1, *H*).

Class 7: Plants with reaction type like class 6, except with five to eight pustules per leaf surface (pl. 1, *I*).

Class 8: Reaction type like classes 6 and 7 except with pustules more numerous, causing the chlorotic areas surrounding the pustules to coalesce, thus producing a more or less uniform chlorotic condition where the pustules were thick (pl. 1, *J*).

Class 9: Pustules on older leaves surrounded by narrow "green islands" with intervening chlorosis, giving the leaves a spotted appearance. Large pustules on younger leaves similar to those in classes 6, 7, and 8. Many pustules per plant (pl. 1, *K*).

Class 10: Reaction type like class 9 except with few pustules per leaf (pl. 1, *L*).

FIELD STUDIES

The rust reaction of the parental varieties grown at Coon Creek during the summers of 1934 and 1935 are presented in table 1.

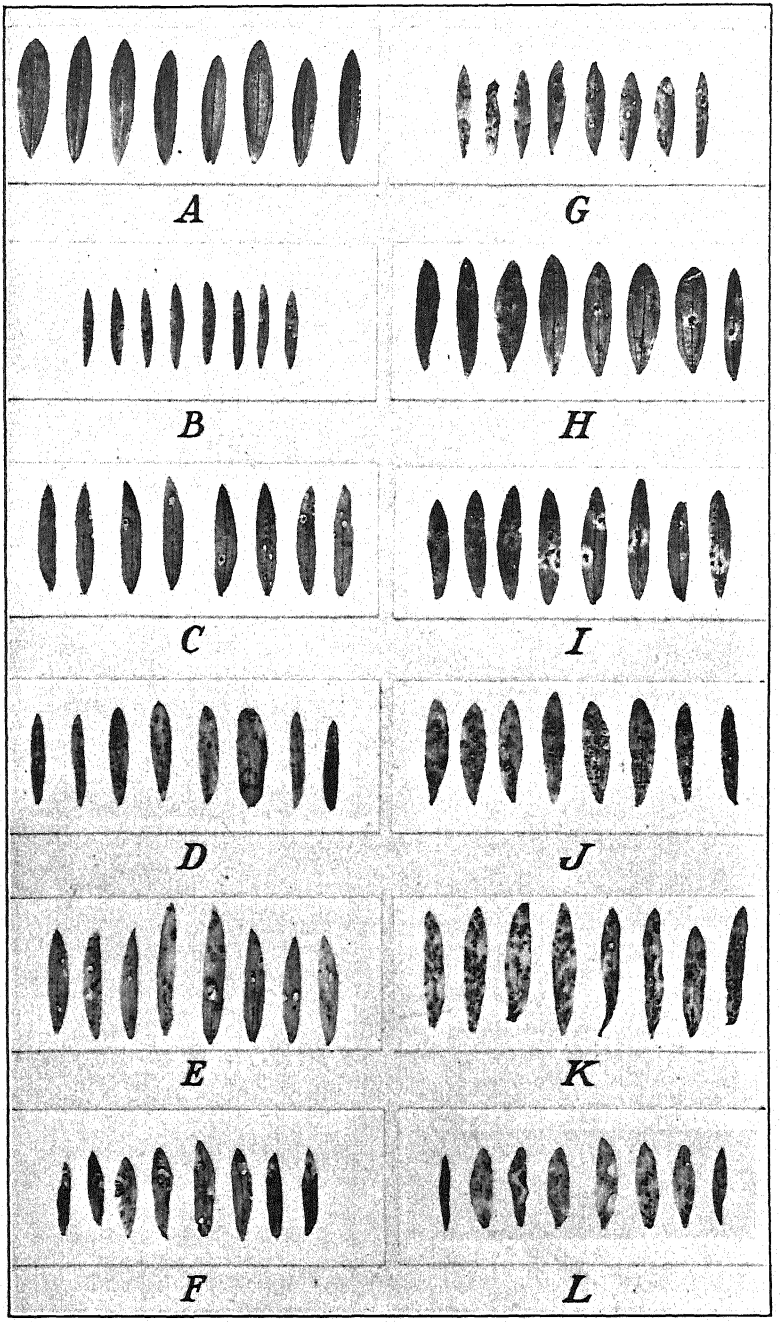
TABLE 1.—*Rust reaction of the parental varieties in the field in 1934 and 1935*

Variety	1934	1935	Variety	1934	1935
Ottawa 770B	Immune	Immune.	C. I. 649	Resistant	Resistant.
Newland	do	Do.	Bison	Semiresistant	Semiresistant.
C. I. 438	do	Do.	Redwing	do	Do.
Long × E	do	Do.	C. I. 391	Moderately susceptible.	Moderately susceptible.
C. I. 416–3	do	Do.	Pale Blue	do	Do.
C. I. 712	do	Do.	Abyssinian Yellow.	do	Do.
Bolley Golden	[1]	Resistant or immune.	C. I. 479	do	Do.
Light Mauve	Immune	Resistant.			

[1] Leaves destroyed; reaction could not be determined.

No rust was found in either year on the six varieties which were considered immune in outlining the experiment, i. e., Ottawa 770B, Newland, C. I. 438, Long × E, C. I. 416–3, and C. I. 712. In addition, all plants of Light Mauve were entirely free from evidence of rust infection in 1934, but in 1935 this variety showed flecks and small resistant pustules. Likewise, a small proportion of the plants of C. I. 649 grown in 1934 were free from rust, the remainder showing only a few resistant-type pustules per plant, while in 1935 occasional large pustules were found along with flecks and resistant pustules on this variety. In 1934, the leaves of Bolley Golden were entirely destroyed by the pasmo disease, making it impossible to determine rust reaction. In 1935 one plant row of Bolley Golden, from the plant used as a parent in the cross C. I. 479 × Bolley Golden, was immune, the remaining plants of Bolley Golden showing a resistant reaction.

Large susceptible-type pustules were found in Bison and Redwing, but in these two varieties the pustule frequency was less than in the four moderately susceptible varieties, C. I. 391, Pale Blue, Abyssinian Yellow, and C. I. 479. Thus the semiresistance of Bison and Redwing under these conditions was a matter of lower pustule frequency rather than type of reaction. Likewise the four moderately susceptible varieties differed from susceptible varieties such as Winona in having a lower percentage of rust rather than in type of pustule. The data on rust reaction in the F_2 generation of Ottawa 770B × Pale Blue and the F_2 and F_3 generations of Ottawa 770B × Redwing grown in the field in 1934 are summarized in table 2.



Classes of rust reaction used in classifying material in the greenhouse studies: *A*, Class 0; *B*, class 1; *C*, class 2 (pustules surrounded by necrosis); *D*, class 2 (pustules surrounded by chlorotic tissue) *E*, class 3; *F*, class 4; *G*, class 5; *H*, class 6; *I*, class 7; *J*, class 8; *K*, class 9; *L*, class 10.

TABLE 2.—*Segregation for rust reaction in the field of F_2 and F_3 generations of crosses involving Ottawa 770B as the immune parent*

Cross	Generation	Plants or lines showing indicated reaction		
		Immune	Segregating	Rusted
		Number	*Number*	*Number*
Ottawa 770B × Redwing	F_2 plants	44		13
Do	F_3 lines	31	47	21
Ottawa 770B × Pale Blue	F_2 plants	45		10

The rusted segregates in Ottawa 770B × Redwing all appeared semiresistant like Redwing. In the F_2 generation the number of plants was small but the fit to a 3:1 ratio was good, P lying between 0.70 and 0.80. The F_3 generation offers more critical evidence regarding the mode of inheritance of immunity. The fit of the observed ratio of F_3 lines to the theoretical 1:2:1 ratio was good, P lying between 0.30 and 0.50. χ^2 for fit to a 3:1 ratio was calculated for each of the 47 segregating F_3 lines separately and the χ^2 values added. The total χ^2 was 38.519. Applying the formula $\sqrt{2\chi^2}-\sqrt{2n-1}$ given by Fisher (*2*) a normal deviate of -0.866 ± 1 was obtained, the deviation from the expected 3:1 ratio not being significant. In the cross Ottawa 770B × Pale Blue, the number of F_2 plants was again too small to warrant drawing definite conclusions although the observed segregates approached the expected 3:1 ratio, with P lying between 0.20 and 0.30. These data are in agreement with the conclusion reached by Henry (*6*) that the immunity of Ottawa 770B in the field is conditioned by a single dominant factor.

GREENHOUSE STUDIES

The major portion of this paper is concerned with the reaction of the parents and hybrids under greenhouse conditions to a collection of rust and to physiologic race 4. It is known that in wheat certain varieties which are very susceptible to rust in the seedling stage in the greenhouse are highly resistant to the same forms of rust in the "mature-plant" stage in the field (Stakman (*8*), Hayes, Stakman, and Aamodt (*4*), and others). Flor (*3*) found that certain varieties of flax showing some resistance in the field were susceptible in the greenhouse. Therefore it seems desirable to know the rust reaction in the greenhouse of the varieties used as parents in this study before attempting to analyze the data obtained on the hybrids. Furthermore, the 11 classes of rust reaction used in classifying the parents and hybrids, were set up to include all of the differences which seemed sufficiently clear-cut to permit their classification, but no attempt was made at that time to determine their genetical significance. Such information, however, is valuable in analyzing the results obtained in segregating populations, and the best evidence of the validity or significance of the different rust classes from a genetical standpoint is to be found in the reaction of different biotypes under the conditions of the experiment.

PARENTAL REACTION

The data on the reaction of the parental varieties to a collection of rust and to form 4 are presented in table 3.

TABLE 3.—*Reaction of plants of parental checks to the collection of rust in the greenhouse and to form 4*

REACTION TO COLLECTION OF RUST IN GREENHOUSE

Variety	Plants in rust class—										
	0	1	2	3	4	5	6	7	8	9	10
	Number	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*
Ottawa 770B	451										
Newland	150										
C. I. 438	113										
Long × E	113	72	14								
C. I. 416–3	123	38									
C. I. 712	34	94	36								
Bolley Golden	66	65	86								
Light Mauve	38	72	94								
C. I. 649		21	15	73		30					
Bison				2	10	81	31	33			4
Redwing						48		56		18	1
C. I. 391						25		35			
Pale Blue							2	120		11	13
C. I. 479							5	45	1	47	38
Abyssinian Yellow								53			3

REACTION TO FORM 4

Ottawa 770B	346										
Newland	145										
C. I. 438	156										
Long × E	123	32	1								
C. I. 416–3	130	35									
C. I. 712	30	80	32								
Bolley Golden	52	63	28								
Light Mauve	32	73	61								
C. I. 649		17	61	42		2					
Bison			5	45		157		2			
Redwing						2		131			
C. I. 391								46			
Pale Blue								181			
C. I. 479								109			
Abyssinian Yellow								67			

All the plants of Ottawa 770B, Newland, and C. I. 438 were immune, both from the collection and from form 4. These varieties had also been immune in the field. Two other varieties, Long × E and C. I. 416–3, immune under field conditions, showed a mixed reaction in the greenhouse.

Such variable or mixed reaction may either have been the result of genetical variability or the influence of environment. Since bulk seed of both these varieties was used in making the crosses, the possibility that the plants tested in each variety did not represent a single biotype cannot be excluded. Four plants of Long × E were used as parents in making the crosses in the greenhouse in 1933–34 and the offspring of one single plant selection from the progeny of each of these four plants were tested both to the collection and to form 4. The data are given in table 4.

TABLE 4.—*Reaction to collection of rust in greenhouse and to form 4 of offspring of single-plant selections from the progeny of plants of Long × E and C. I. 712 used as parents*

LONG × E

1933–34, plant no.	1934, no.	1935, no.	Plants in rust class indicated when inoculated with—						
			Collection				Form 4		
			0	1	2	3	0	1	2
			Number	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*
132–1	4358–1	6107–2	16	2			12	4	
132–2	4367–1		5	12	3		1	12	1
133–4	4377–1	6189–1	5	7			30	7	
134–1	4373–1		20				14		
			13	3			12		

C. I. 712

140–1	4340–1	6005–1	5	7			3	12	
	4365–3		9	12			10	7	
140–4	4368–1			7		2		15	3
								14	4
141–1	4352–1					13		6	14
				3		16		9	11
142–2	4362–1			13		1			

The progeny of the two plants 132–1 and 134–1 were predominantly class 0, whereas the progeny of 132–2 were mostly class 1, both to the collection and to form 4. These results, although not conclusive, indicate that two different strains of Long × E had been used. This does not answer the question, however, whether the occurrence of both class 0 and class 1 progenies from the same plant resulted from heterozygosity for factors conditioning rust reaction or from the influence of environment. In a naturally self-pollinated crop like flax, the proportion of plants heterozygous for any given factor or factors, after the variety has been grown for several years, must be low. Therefore, it is unusual that all four of the plants used in the greenhouse should have been heterozygous and, in addition, that the single plant selected from their progeny should, in each of the four cases, also have been heterozygous.

Four plants of C. I. 416–3 were used as parents in the crosses made in the greenhouse. From the progeny of a single plant selected from the offspring of one of these original plants, three plant selections were made, the progeny of which were used as checks in the greenhouse rust tests. Likewise two plants from the progeny of a single plant selected from offspring of another original parent plant and single-plant selections from the progeny of the other two original parents were taken, the progeny of which were used as checks in the greenhouse tests. No sharp differences in the rust reaction of the different parental lines were observed. The greatest difference was between two pots planted with seed of a single plant. The 18 plants in one pot, inoculated October 13, were all class 0, while the 19 plants in the other pot, inoculated on November 25, were all class 1. These results indicate that in this variety the class 0 and class 1 types of reaction are subject to environmental fluctuations to a considerable extent.

The complexity of studies of disease resistance has been pointed out by other investigators. Reaction of the host to a disease is not merely the interaction of the genetic factors of a single organism with the environment but rather the interaction of two organisms, the host and the pathogen, each with its gene complex interacting with the environmental conditions. If immunity in flax is dependent upon the limitation of the development of the pathogen rather than its exclusion, it would perhaps not be surprising to find cases in which the balance between host and pathogen is so delicate that slight changes in the environment shift the reaction from one in which no macroscopic disease symptoms occur to one in which sufficient host cells are killed to produce a fleck.

C. I. 712, which was immune in the field, when tested in the greenhouse had plants in the three rust classes, 0, 1, and 2. Bulk seed of this variety had also been used for making the original crosses. Four plants were used as parents for the crosses made in 1933–34. The data on rust reaction of lines descending from these four plants are summarized in table 4.

Offspring from the plant selections from the progeny of 140–1 consist of plants in rust classes 0 and 1, while progeny of the other three lines have all plants in classes 1 and 2.

Two plants of Bolley Golden were used as parents in the greenhouse in 1933–34. Sixty-six plants of the line descendant from plant 123–2 were inoculated with the collection of rust in the greenhouse and all were immune. In addition, 32 plants of this line were inoculated with form 4 with the same results. This is the same line that was immune in the field. Progeny from four single-plant selections from offspring of the other parent plant varied in rust reaction from class 1 to class 2. Four pots were planted with seed from one of these single-plant selections and inoculated with the collection of rust. Plants in two of these pots fell in class 1, whereas those in the other two pots, inoculated at a later date, were classed as 2, i. e., with resistant-type pustules. This indicates that classes 1 and 2 may represent environmental fluctuations rather than true genetical differences, in this variety at least. In 1934, three plants of Bolley Golden were used in making additional crosses. Progeny of one of these plants were all class 0 while the progeny of the other two varied in rust reaction from class 1 to 2. It is probable that the variety Bolley Golden consists of a mixture of immune and resistant biotypes. Flor (*3*) found that 70 percent of the plants of Bolley Golden were immune, and 30 percent were resistant in his tests.

A similar situation obtained in Light Mauve. Thirty-nine individuals from a single-plant selection of the progeny of one of the parent plants used in making the crosses in the greenhouse were inoculated with the collection of rust. Thirty-six were class 0 and three were class 1. Eighteen plants of the same line were inoculated with form 4 and all were class 0. Progenies of plant selections from descendants of the other three original plants, when inoculated with the collection, were all classed as 1 or 2 except two, which were class 0. These two may have been escapes. When inoculated with form 4, all were placed in classes 1 and 2. No distinct differences in rust reaction between the descendants of these three original plants were observed. Three plants of Light Mauve were used in making additional crosses in 1934. Progenies of two of these, when tested with the collection of rust,

were classed as 1 and 2. Progenies of all three plants when tested with form 4 contained class 0, 1, and 2 plants. Whether the plants in class 0 represented true immunity or escapes is not known.

The plants of C. I. 649 varied from class 1 to class 5 both when inoculated with the collection and when inoculated with form 4. The proportion of class 1 plants was greater, and that of class 3 and 5 plants smaller, among the plants inoculated with form 4 than among those inoculated with the collection. The range in rust reaction from class 1 to class 5 is greater than would normally be expected in plants of the same genotype, particularly on such plants growing in the same pot. However, the seed of C. I. 649, used in making the greenhouse crosses, was taken from a single-plant selection, and the progeny of five single-plant selections from the offspring of two of the original parent plants gave, in each case, a similar range in reaction types. These results would suggest homozygosity of the material, although the only critical evidence would be a progeny test of individuals representing the four reaction classes. Such tests have not as yet been made. Marquillo wheat is known to show variability in reaction, giving both resistant and semiresistant plants (Hayes, Stakman, and Aamodt (*4*), Ausemus (*1*). Whether this is an analogous situation is not known.

The plants of Bison, inoculated with the collection of rust, varied from class 3 to 10, while the plants inoculated with form 4 were placed in classes 2, 3, 5, and 7. These results indicate that Bison is likewise more resistant to form 4 than to the collection. Flor (*3*) did not find such a difference either with C. I. 649 or Bison. Since, in the present study, the varieties were inoculated with form 4 and the collection at different times; it is not certain that a true difference in resistance obtains. Although the range in rust reaction among the Bison plants was large, a study of the progenies of the individual plant selections afforded no evidence regarding the nature of this variation. Classes 4 and 5 have a similar type of reaction and differ only in the pustule frequency. Class 4 was found in the early inoculations, but later all plants showing this type of pustule fell in class 5, perhaps due to a more uniform distribution of inoculum as the inoculation technique improved. A similar situation obtained for classes 6, 7, and 8, where the relative proportion of class 7 plants increased not only in Bison but also in other varieties to be discussed later, as the experiment progressed.

Plants of Redwing, inoculated with the collection, occurred in classes 5, 7, 9, and 10, while C. I. 391 had plants in classes 5 and 7. Class 5 plants were found in Redwing early in the experiment, while all plants inoculated later, under reduced intensity and duration of light, were classed as 7. In both of these varieties, when inoculated with form 4, all plants were placed in class 7 except two plants of Redwing in class 5.

Plants of Pale Blue and C. I. 479 varied from class 6 to class 10 when inoculated with the collection. In the early part of the investigation, the "green island" type of reaction of classes 9 and 10 occurred in the same pots of these two varieties along with classes 6, 7, and 8. Later, with reduced light, this type of reaction no longer appeared. All plants of these two varieties, when inoculated with form 4, gave a uniform class 7 type of reaction. It is doubtful from the results ob-

tained with these two varieties whether classes 6, 7, 8, 9, and 10 represent true genetical differences.

On the basis of the above results, it appeared logical to place the parents in five groups, as follows:

(1) Immune: Ottawa 770B, Newland, C. I. 438, and the immune strain of Bolley Golden.
(2) Near immune: Long × E, C. I. 416–3, and the near-immune strain of Light Mauve.
(3) Resistant: Bolley Golden, Light Mauve, and C. I. 712.
(4) Semiresistant: Bison, Redwing, and C. I. 391.
(5) Susceptible: Pale Blue, C. I. 479, and Abyssinian Yellow.

C. I. 649 is less resistant than Bolley Golden, Light Mauve, or C. I. 712. On the other hand, it is definitely more resistant than the varieties classed as semiresistant. Because of the variable reaction of the plants of C. I. 649 in this study, this variety has not been placed in any of the five groups.

C. I. 649 was used in crosses with one immune variety, Ottawa 770B, one resistant variety, Light Mauve, and three semiresistant varieties, Bison, Redwing, and C. I. 391.

On the basis of the rust reaction of the parents under greenhouse conditions, the crosses can be classified as those involving parents with similar rust reaction and those involving parents with different rust reaction, as follows:

Crosses involving parents with similar rust reactions:

Immune × immune: Ottawa 770B × Newland, Ottawa 770B × C. I. 438, and C. I. 438 × Newland.
Near immune × near immune: C. I. 416–3 × (Long × E).
Semiresistant × semiresistant: Redwing × Bison.
Susceptible × susceptible: C. I. 479 × Pale Blue and Pale Blue × Abyssinian Yellow.

Crosses involving varieties with different reaction to rust:

Immune × near immune: Ottawa 770B × C. I. 416–3, Ottawa 770B × (Long × E), C. I. 416–3 × Newland, C. I. 416–3 × C. I. 438, C. I. 438 × (Long × E), and (Long × E) × Newland.
Immune × resistant: Ottawa 770B × Bolley Golden, Ottawa 770B × Light Mauve, Ottawa 770B × C. I. 712, C. I. 712 × Newland, and C. I. 712 × C. I. 438.
Immune × semiresistant: Ottawa 770B × Redwing and Ottawa 770B × Bison.
Immune × susceptible: Ottawa 770B × Pale Blue, Ottawa 770B × C. I. 479, and C. I. 479 × Bolley Golden.
Near immune × resistant: C. I. 712 × (Long × E) and C. I. 712 × C. I. 416–3.
Near immune × semiresistant: Light Mauve × Redwing.
Resistant × semiresistant: Light Mauve × Bison and C. I. 391 × Light Mauve.
Resistant × susceptible: Bolley Golden × Pale Blue and Bolley Golden × Abyssinian Yellow.
Semiresistant × susceptible: Pale Blue × Bison and C. I. 479 × Bison.

REACTION OF HYBRID MATERIAL

Immune × Immune

In the cross of Ottawa 770B × C. I. 438, 32 F_3 lines, averaging 15.6 plants per line, were inoculated with the collection of rust and only class 0 plants were obtained. Inoculation of these same F_3 lines with form 4 gave identical results. The results indicate that the immunity of Ottawa 770B is allelic to the immunity of C. I. 438.

The data on the reaction to the collection of rust of parents, F_1, and F_3 of Ottawa 770B × Newland and the parents and F_3 generation of C. I. 438 × Newland are presented in table 5.

TABLE 5.—*Reaction of parents, and of F_1 and F_3 generations of Ottawa 770B × Newland and C. I. 438 × Newland to collection of rust in greenhouse*

Parent or generation	Number of plants or lines showing indicated reaction[1]						
	I	I, R	I, SR	I, SR, S	I, S	R	S
Ottawa 770B ---- plants	34						
Newland ---- do	30						
F_1 ---- do	5						
F_3 ---- lines	20		1	2	11		1
C. I. 438 ---- plants	31						
Newland ---- do	32						
F_3 ---- lines	16	13				1	

[1] I=immune, R=resistant, SR=semiresistant, S=susceptible.

In the 35 F_3 lines of Ottawa 770B × Newland, semiresistant (class 5) and susceptible segregates were obtained in addition to class 0 plants. Grouping the class 5 and susceptible segregates together, the ratio of F_3 lines becomes 20 immune : 14 segregating : 1 susceptible. Using χ^2 to compare this ratio with the calculated, on the basis of duplicate factors for immunity, gave a value of P between 0.10 and 0.20. These data suggest the hypothesis of a single dominant factor conditioning immunity in each variety the two factors not being allelic and inherited independently.

The relationship of the reaction of these lines to the collection and to form 4 is shown in table 6.

TABLE 6.—*Correlation table showing the reaction of F_3 lines of Ottawa 770B × Newland to collection of rust in greenhouse and to form 4*

Reaction to form 4	Reaction to collection			
	Immune	Segregating	Susceptible	Total
	Number	*Number*	*Number*	*Number*
Immune	20	5		25
Segregating		8		8
Susceptible		1	1	2
Total	20	14	1	35

From these data it can be seen that the reaction of the F_3 lines to form 4 was similar to their reaction when inoculated with the collection. Five lines that were classed as segregating with the collection contained only class 0 plants when inoculated with form 4. However, since the average number of plants per line was only 11.5 in the test with form 4, these exceptions probably resulted from failure to recover recessive segregates in some lines owing to the small number of plants. In addition, one line was classed as segregating with the collection and susceptible to form 4. In this line only three plants were tested in each case.

In C. I. 438 × Newland (table 5), 16 of the F_3 lines contained only class 0 plants, 13 contained, in addition to class 0, class 1, 2, or 3 segregates and 1 line contained only class 1, 2, and 3 plants. The occurrence of class 1, 2, and 3 plants in these F_3 lines suggests the absence of allelism of the factor for immunity in Newland with a factor conditioning immunity in C. I. 438. χ^2 for fit of observed to calculated on the basis of two dominant factors for immunity gave a P value between 0.20 and 0.30. Since no susceptible segregates were obtained in the F_3 lines, it appears that one of the varieties carries, in addition to a factor for immunity, a factor for resistance allelic to the factor conditioning immunity in the other variety. No evidence of a factor determining resistance was found in the cross of Ottawa 770B × Newland. Therefore, it seems probable that C. I. 438 carries at least two factors, one conditioning immunity and the other, which is allelic to the factor for immunity in Newland, conditioning resistance to the collection of rust. When the F_3 lines of C. I. 438 × Newland were inoculated with form 4, all plants were immune. This indicates that the factor in C. I. 438 which conditions resistance to the collection determines immunity from form 4.

Near Immune × Near Immune

In C. I. 416–3 × (Long × E), 25 F_3 lines, averaging 12.7 plants per line, were tested with the collection of rust. Only class 0, 1, and 2 plants were obtained. The variability of the two parents from class 0 to 1 made an exact genetical interpretation of the results in the F_3 lines impossible. However, the absence of semiresistant or susceptible segregates in any of the F_3 families indicates that the near immunity of these two varieties are allelic. Likewise, only class 0, 1, and 2 plants were obtained when these lines were inoculated with form 4.

Semiresistant × Semiresistant

The data on the reaction of parents, F_1, and F_3 generations of Redwing × Bison to the collection and to form 4 are given in table 7.

Of the 32 F_3 lines inoculated with the collection, 14 contained only class 4 and 5 plants, while the remaining 18 contained both class 4 and 5 and susceptible segregates. The reaction of Bison and Redwing is variable, as pointed out in the analysis of the parental reaction in the greenhouse, thus precluding the formulation of any factorial explanation of the results obtained in this cross.

The results with form 4 differ somewhat from those obtained with the collection in this cross. One of the plants of Redwing was placed in class 5 and 28 in class 7, whereas the Bison plants were placed in classes 3, 5, and 7. Here again a factorial explanation of the results in F_3 cannot be given. It is interesting to note that the proportion of class 3 segregates in the material inoculated with form 4 is greater in those lines showing only class 4 and 5 segregates when inoculated with the collection. Likewise, the proportion of plants showing a susceptible reaction to form 4 is greater in those lines showing susceptible segregates with the collection of rust.

TABLE 7.—*Reaction of parents, and of F_1 and F_3 generations of Redwing × Bison to collection of rust in greenhouse and to form 4*

Parent or generation	Plants in rust class indicated when inoculated with—									
	Collection							Form 4		
	4	5	6	7	8	9	10	3	5	7
	Number	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*
Redwing		30					1		1	28
Bison		32						5	29	1
F_1		5							5	
F_3 families:										
6011-7		16						5	7	
6011-13		17							11	4
6011-15		15							10	6
6011-16		15						1	14	
6011-20		20							13	4
6011-21		14							9	4
6011-27		3						2	6	6
6011-28		20						3	14	
6011-31		16						10		
6011-8	3	9						2	7	
6011-10	9	4						2	7	5
6011-12	1	16						6	8	
6011-17	1	17							13	
6011-30	4	12						4	10	
6011-5	4	10		2				2	8	4
6011-2		6					6			16
6011-3		9					3	1	7	7
6011-4		8					6			10
6011-6		16					3			14
6011-19		12					3		3	8
6011-22		11					1	2	9	5
6011-23		17					1	1	14	
6011-24		14					2		13	5
6011-25		9					6	1	10	6
6011-11		8		3			6		12	5
6011-18		9		1			3		6	10
6011-29		5		4			9		1	17
6011-9		4				3	6		10	5
6011-32		11				1	3		12	4
6011-1		9		1		3	1	1	7	5
6011-14		2			1	11	1			18
6011-26		4	1	2	1		3		11	

SUSCEPTIBLE × SUSCEPTIBLE

In the cross Pale Blue × Abyssinian Yellow, all plants of both parents and the F_1 were classed as susceptible in the material inoculated with the collection, while in the 30 F_3 lines, 3 contained only class 5 plants, 19 contained class 5 and susceptible plants, and 8 contained only susceptible segregates. On the basis of the results obtained in Bison, Redwing, and C. I. 391, the varieties showing some semiresistant plants, it is evident that the class 4 and 5 type of reaction is not very sharply differentiated genetically from susceptible. Consequently, it is possible that the class 5 plants obtained among the progeny of this cross were merely fluctuations caused by environment. However, the absence of such plants in either parental variety suggests a true genetical difference, although it is impossible to give a factional explanation of the results. When this cross was inoculated with form 4, all plants of the parents, F_1 and F_3 lines were susceptible.

In the cross C. I. 479 × Pale Blue, all plants of the parents, F_1, and of 63 F_3 lines were susceptible both to the collection and to form 4.

Immune × Near Immune

The data on the reaction of the parents, F_1, and F_3 lines of Ottawa 770B × C. I. 416–3 are presented in table 8.

Table 8.—*Reaction of parents, and of F_1 and F_3 generations of Ottawa 770B × 416–3 to collection of rust in greenhouse and to form 4*

Parent or generation	Plants in rust class indicated when inoculated with—									
	Collection					Form 4				
	0	1	2	3	7	0	1	2	3	7
	Number	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*
Ottawa 770B	21					39				
C. I. 416-3		34				34	6			
F_1	1									
F_3 lines:										
4212-4	17					19				
4212-12	18					19				
4212-22	20					16				
4212-27	20					20				
4212-33	20					19				
4212-37	10					19				
4212-2	15	4				18				
4212-6	12	5				15	1			1
4212-10	19	1				14				
4212-11	12	3				18				1
4212-14	8	3				18				
4212-19	15	2				17				
4212-23	17	3				16	3			
4212-24	15	3				10	4			
4212-28	16	4				18				
4212-30	18	2				17	2			
4212-32	15	2				16	1	1		
4212-36	10	4								
4212-15	9	6				16				
4212-21	5	12	1			17	1			
4212-7	17				2	13	1	1		2
4212-13	9				3	17				
4212-18	16				4	13	7			1
4212-38	11				3	13		2		2
4212-11	12	3			1	18				1
4212-29	14	1		2	3	16				3
4212-3	1	17			2		16			3
4212-25		16	2	2		14	2			
4212-31		17	1	1		10	4	1		4
4212 8		20				18	2			
4212-17	1			2	15					20
4212-26				5	13	1		9	4	6

In the material inoculated with the collection, 2 of the 32 F_3 lines were predominantly susceptible although 2 plants out of the 18 in 1 line and 5 out of 18 in the other were placed in class 3. The single class 0 plant in one of these two lines may have been a natural hybrid. The occurrence of two susceptible lines and of susceptible segregates in seven other lines indicates that the near immunity of C. I. 416–3 is not allelic to the immunity of Ottawa 770B. Although the number of lines showing susceptible segregates was less than would be expected, the results in this cross could be explained on the hypothesis of two dominant factors, one conditioning the immunity of Ottawa 770B and the other determining the near immunity of C. I. 416–3. However, since the mean number of plants in the F_3 lines was 17.56, the numbers are too small to warrant definite conclusions regarding the number of factors involved.

TABLE 9.—*Correlation table showing the reaction of F_3 lines of Ottawa 770B × C. I. 416–3 to collection of rust in greenhouse and to form 4*

Reaction to form 4	Reaction to collection			
	Immune, near immune	Segregating	Susceptible	Total
	Number	*Number*	*Number*	*Number*
Immune, near immune	19	1		20
Segregating	3	6	1	10
Susceptible			1	1
Total	22	7	2	31

The relation of the reaction of the F_3 lines to the collection and their reaction to form 4 is shown in table 9. Since classes 0, 1, 2, and 3 do not appear to be differentiated by major genetic factors in this cross, these four classes are combined in this case. The results indicate that the major factors differentiating the reaction of the F_3 lines to the collection of rust are the same as those conditioning reaction to form 4.

In the material of Ottawa 770B × (Long × E), inoculated with the collection, 31 F_3 lines, averaging 15 plants per line, were studied and only class 0, 1, and 2 segregates were obtained, indicating that the near immunity of Long × E is allelic to the immunity of Ottawa 770B. The variability of the near immune reaction of Long × E made an exact factorial explanation of the results in this cross impossible. When these F_3 lines were inoculated with form 4, only class 0, 1, and 2 plants were again obtained.

In the 35 F_3 lines of (Long × E) × Newland, no semiresistant or susceptible segregates were obtained either in the material inoculated with the collection or with form 4, indicating that the factor conditioning immunity in Newland is allelic to a factor for near immunity in Long × E. A similar result was obtained in inoculations both with the collection and the single form of 31 F_3 lines of C. I. 416–3 × Newland, likewise suggesting allelism of the near immunity of C. I. 416–3 with the immunity of Newland.

The data on the reaction of parents and F_3 families of C. I. 438 × (Long × E) are presented in table 10.

In the material tested with the collection, the occurrence of 6 F_3 lines out of 33 with class 5 and susceptible segregates suggests the possibility of genetical segregation. It is interesting to note that these segregates all occur in the progeny of one F_2 family, 5163. These results indicate that the two F_1 plants from which F_2 families 5163 and 6166 descended were not alike genetically. This perhaps resulted from the use of a heterozygous parent in making the cross. As can be seen in table 10, the behavior of the F_3 lines when tested with form 4 was similar to their reaction to the collection.

Of the 32 F_3 families of C. I. 416–3 × C. I. 438 inoculated with the collection of rust, all the plants in 31 lines were class 0, and in the other family 10 plants were class 0 and 1 plant was class 1. Also, all plants of C. I. 416–3, inoculated as checks with this cross, were class 0. The average number of plants in the F_3 lines in this cross was 12.75. When inoculated with form 4, all plants of C. I. 416–3 and of the

32 F_3 families were class 0. From these results it appears that the near immunity of C. I. 416–3 is allelic to the immunity of C. I. 438.

TABLE 10.—*Reaction of parents and of F_3 generation of C. I. 438 × (Long × E) to collection of rust in greenhouse and to form 4*

Parents or generation	Plants in rust class indicated when inoculated with—								
	Collection						Form 4		
	0	1	2	3	5	7	0	1	7
	Number	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*
C. I. 438	30						39		
(Long × E)	33	3					26		
F_3 Families:									
6166-1	12						1		
6166-7	24						9		
6166-9	34						16		
6166-10	31						11		
6166-12	19								
6166-14	18						7		
6166-16	22						4		
6166-17	28						7		
6166-19	16						1		
5163-4	12						7		
5163-11	19						3		
5163-13	15						2		
6166-6	35	1					11		
6166-8	21	4							
6166-13	23	1					12		
6165-1	14	2							
6166-3	21	1					16	1	
6166-5	16	1					8	2	
6166-20	19	3					7		
5163-12	12	1					7		
5163-14	18	2					7		
5163-19	26	1					8		
5163-20	29	3					8		
6166-11	21	3	1				17		
6165-18	25	5	4						
6166-21	29	5	1				4	1	
5163-6	12			2			11		1
5163-8	17	1			1		4		1
5163-16	27		1		3		11		1
5163-17	15	3			1	1	13		
5163-18	25	3			1	1	10		
5163-22	4	4			3	1	5		2
5163-2	15	1				4	7	2	

IMMUNE × RESISTANT

In the crosses involving Ottawa 770B with Bolley Golden and Light Mauve, only F_2 generations were studied; the data for these crosses are presented in table 11.

TABLE 11.—*Reaction of parents and F_2 generation of crosses of immune × resistant varieties to collection of rust in greenhouse*

Parent or generation	Plants showing indicated reaction						
	0	1	2	3	5	7	9
	Number	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*
Ottawa 770B	75						
Bolley Golden		36	37				
F_2	448	32	70		4	24	4
Ottawa 770B	38						
Ligh Mauve	2	33	5				
F_2	169	2	8	3	5		1
Ottawa 770B	37						
Light Mauve		4	33				
F_2	294	1	51	6	5	16	

The plants of Bolley Golden occurred in the two classes 1 and 2. As pointed out in the discussion of parental reaction in the greenhouse, it seems probable that these two classes represent fluctuations due to environment rather than actual genetical differences in this variety. Likewise, the data on parental reaction indicated that classes 6, 7, 8, 9, and 10 did not represent true genetical differences, but rather variations due to environment. Consequently, the analysis of the results in segregating populations would be facilitated by combining class 1 and 2 into a resistant group and the last five classes into a susceptible group.

On this basis, the F_2 plants of Ottawa 770B × Bolley Golden occur in the ratio of 448 immune:102 resistant:4 class 5:28 susceptible. Considering the class 5 and susceptible segregates together, χ^2 for fit of the observed ratio to the theoretical 12:3:1 ratio gave a value of P between 0.50 and 0.70. Such a result could be explained on the assumption of a single dominant factor conditioning the immunity of Ottawa 770B and a single dominant factor for resistance carried by Bolley Golden which is not allelic but hypostatic to the factor for immunity contributed by Ottawa 770B. The validity of combining the four class 5 segregates with the susceptible group may be subject to question. However, these plants are definitely more susceptible than the resistant group of segregates, which resemble Bolley Golden in their rust reaction.

In Ottawa 770B × Light Mauve, a ratio of 169 immune:10 resistant:3 class 3:5 class 5:1 susceptible was obtained. Class 3 apparently represents a type of resistance and differs from class 2 of the resistant group only in having occasional large pustules in addition to the flecks and resistant type pustules. Combining the class 3 plants with the resistant group and again considering the class 5 and susceptible segregates together, a ratio of 169:13:6 obtains. χ^2 for fit of this ratio to a 12:3:1 ratio is 22.418, a value greatly in excess of χ^2 for the 1 percent point. The deviation from the expected ratio results from an excess of immune segregates and a deficiency particularly in the resistant class. Since, in this case, 2 of the 40 Light Mauve plants were classed as immune, the excess of class 0 segregates may have been due to escapes. To test this possibility, additional F_2 plants of this cross were inoculated. In the second trial, a ratio of 294 immune:52 resistant:6 class 3:5 class 5:16 susceptible segregates obtained. Again combining class 3 with the resistant group and class 5 with the susceptible group, χ^2 for fit of observed to a 12:3:1 ratio gave a value of P between 0.20 and 0.30. These results indicate that Light Mauve carries a single dominant factor for resistance which is not allelic but hypostatic to the factor conditioning immunity in Ottawa 770B.

In Ottawa 770B × C. I. 712, the F_1 plants were all immune both when tested with the collection and with form 4. The results in the 30 F_3 lines are summarized in table 12. In this case the class 0 plants were considered immune and the class 1, 2, and 3 segregates were again combined into a resistant group. Considering the resistant and semiresistant segregates together in the material inoculated with the collection, the observed ratio of 7 immune:14 segregating:9 not immune lines compared with the theoretical 1:2:1 ratio by means of χ^2, gave a value of P between 0.80 and 0.90. The reaction of the

F_3 lines to form 4 was similar to their reaction to the collection. However, two lines which showed a resistant reaction to the collection had mostly class 0 plants when inoculated with form 4. In addition, one line was predominantly class 0 with the collection, but when tested with form 4, seven of the eight plants were in class 1. It seems probable that these exceptions may be the result of environmental fluctuations rather than true genetical differences. Semiresistant segregates in two lines both when inoculated with the collection and with form 4, and the susceptible segregates in two lines when tested with form 4, may have resulted from the lack of allelism of the factors for resistance of C. I. 712 and the factor for immunity in Ottawa 770B or from the segregation of minor modifying factors. No evidence was available in this cross for determining which alternative is correct.

TABLE 12.—*Correlation table showing the reaction*[1] *of F_3 lines of Ottawa 770 B × C. I. 712 to collection of rust in greenhouse and to form 4*

Reaction to form 4	Reaction to collection—				
	I	I, R	I, SR	R	Total
	Number	*Number*	*Number*	*Number*	*Number*
I	6	5			11
I, R	1	6		2	9
I, SR			1		1
I, SR, S			1		1
I, R, S		1			1
R				7	7
Total	7	12	2	9	30

[1] See footnote 1, table 5.

Only class 0, 1, and 2, and 3 segregates were obtained in the 29 F_3 lines of C. I. 712 × C. I. 438 when inoculated with the collection. Combining classes 1, 2, and 3 into a resistant group, the ratio of F_3 lines was 10 immune:13 segregating:6 resistant. χ^2 for fit of this ratio to a 1:2:1 gave a value of P between 0.30 and 0.50. Seed was available for testing with form 4 of only 26 of the F_3 lines of this cross, and, of these, 14 contained only class 0 plants, 11 contained predominantly class 0 with class 1, 2, or 3 segregates in addition, and 1 line contained predominantly class 1 and 2 plants with 1 class 0 plant. This single line seemed to resemble C. I. 712 in reaction to rust. These results are similar to those obtained with C. I. 438 × Newland, and may be explained by assuming that C. I. 438 carries at least two factors for rust reaction, one conditioning immunity from both the collection and form 4, the other conditioning resistance to the collection and immunity from form 4.

The data on C. I. 712 × Newland are presented in table 13.

Of the 31 F_3 families of this cross, 18 contained susceptible or class 5 segregates, or both susceptible and class 5 plants when tested with the collection. The occurrence of these class 5 and susceptible segregates indicates that the resistance of C. I. 712 is not allelic to the immunity of Newland. Further, the proportion of lines showing sus-

ceptible segregates suggests that not more than two pairs of factors were involved. Considering the class 1, 2, 3, 5, and the susceptible plants together, the ratio of F_3 lines was 7 immune: 14 segregating : 10 not immune. Two of the lines classed as not immune each contained a single class 0 plant, but, since immunity is dominant, it is improbable that these plants resulted from genetical segregation. The fit of this observed ratio to a 1:2:1 is good, P lying between 0.50 and 0.70. Total χ^2 for goodness of fit to a 3:1 ratio within the segregating F_3 lines gave P between 0.30 and 0.50. These results are in agreement with the assumption of a single dominant factor conditioning the immunity of Newland. The reaction of the F_3 lines to form 4 seems, in general, to be similar to their reaction to the collection, particularly when the small number of plants per line is considered.

TABLE 13.—*Reaction of parents and of F_1 and F_3 generations of C. I. 712 × Newland to collection of rust in greenhouse and to form 4*

Parent or generation	Plants in rust class indicated when inoculated with—											
	Collection						Form 4					
	0	1	2	3	5	7	0	1	2	3	5	7
	No.	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*
Newland	32						38					
C. I. 712		18	3									
F_1	2											
F_3 families:												
6154–1	9						1					
6154–6	4											
6154–8	13						6					
6154–9	20						19					
6154–14	11						10					
5151–3	7						3	1				
5151–9	9						4					
6154–4	6				2		11	1		2	1	1
6154–5	8					3	1		1			
6154–2	7		1	3			10					
6154–22	5		1	1			7		4		3	
6154–3	6	1				1	9	1		1	1	
6154–10	5	1				1	7		1	1		
6154–12	12	1				3	12				4	
6154–17	5		1			2						
5151–11	7	2				5	6					
5151–16	8			1		2	4			1	1	
5151–6	3				3	1	1				1	
6154–7	11		2	1		1	6					
6154–16	8	1		1		1						
6154–13	6			1	3	3	1		2			
6154–11		6	1						2			
6154–23		7					11	1	2			
5151–14		4	2					2	4			
6154–15	1		3	1								
6154–19		5	4	4		1		2	1	4	5	1
6154–20		1	3	3	6	5		3	4	2	4	
5151–2		13		1	3	1	3	2	6	3		
5151–5		2	1	2	2	1				4		
5151–8	1	2	1		4	2			1	1	5	
5151–10				5	1		2		2			

IMMUNE × SEMIRESISTANT

In the cross Ottawa 770B × Redwing the two F_1 plants were immune like Ottawa 770B, while the plants of Redwing were placed in classes 5 and 9. The data on the reaction of 63 F_3 lines to the collection and to form 4 are summarized in table 14.

TABLE 14.—*Correlation table showing the reaction*[1] *of F_3 lines of Ottawa 770B × Redwing to collection of rust in greenhouse and to form 4*

Reaction to form 4	Reaction to collection—						
	I	I, SR, S	I, SR	IS	SR, S	S	Total
	Number	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*
I	15			1			16
I, SR, S		4		2			6
I, S		7	1	19			27
SR, S					2		2
S					7	5	12
Total	15	11	1	22	9	5	63

[1] See footnote 1, table 5.

The variability of the Redwing parent, in which both semiresistant (class 5) and susceptible plants occurred, illustrates the difficulty of formulating a genetical explanation of the occurrence of the semiresistant segregates. Considering the semiresistant and susceptible segregates together in the material inoculated with the collection, the ratio of the 63 F_3 lines was 15 immune: 34 segregating: 14 not immune. A comparison of this ratio with a 1:2:1 ratio by means of χ^2 gave a P value of 0.80 to 0.90. Total χ^2 for fit to a 3:1 ratio within the segregating F_3 families was 25.171 with 34 degrees of freedom. The normal deviate was -1.09 ± 1, indicating that the deviation from the expected within the segregating F_3 families was within the errors of random sampling.

The principal differences between the reaction to the collection and to form 4 of these 63 F_3 lines were found in the semiresistant and susceptible classes. Since the class 4 and 5 type of reaction was variable these differences may have represented variations due to environment. Considering the immune as contrasted to the not immune segregates, the agreement of reaction to the collection and to form 4 was nearly complete. One line which was segregating with the collection was classed as immune from form 4. These results indicate that the factor conditioning immunity from the collection likewise conditions immunity from form 4.

The data on the reaction of the F_3 families of Ottawa 770B × Bison are given in table 15.

No check plants of either parent inoculated at the same time as the hybrid material were available in studying this cross. Immune, class 5, and susceptible segregates occurred in the 30 F_3 lines when inoculated with the collection Here again it is impossible to offer a factorial explanation for the occurrence of class 5 segregates. Combining the class 5 and susceptible segregates, the ratio of F_3 lines was 10 immune : 9 segregating : 11 not immune. χ^2 for fit to a 1 : 2 : 1 ratio gave a value of P between 0.05 and .010 Total χ^2 for fit to a 3 : 1 ratio within the segregating F_3 lines gave a P value between 0.70 and 0.80, a good fit of observed to calculated. The reaction of the F_3 lines of this cross to form 4 indicates that the factor conditioning immunity from the collection likewise conditions immunity from form 4. When these F_3 lines are inoculated with form 4, class 2 and a number of class 3 segregates occurred. Likewise, Bison, in other tests, contained

some class 2 and class 3 plants when inoculated with form 4. No factorial explanation of these results seems possible.

TABLE 15.—*Reaction of F_3 families of Ottawa 770B × Bison to a collection of rust in greenhouse and to form 4*

Parent or generation	Plants in rust class indicated when inoculated with—									
	Collection					Form 4				
	0	5	7	8	9	0	2	3	5	7
F_3 families:	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*
6148–1	16					15				
6148–4	18					13				
6148–5	18					19				
6148–6	6					14				
6148–9	14					17				
6148–16	17					12				1
6148–22	3					4				
6148–24	11					12				
5145–1	9					8				
5145–2	17					19				1
5145–3	7	1				4				4
6148–3	16			5		15		1	1	
6148–7	13		2	2		10		3	2	1
6148–8	13			2		16		2		1
6148–11	13			6		12				6
6148–18	13			4		12		1	3	1
6148–20	3			2		3			1	
5145–12	7		1			2	3			
5145–17	7			6		8			3	2
6148–2	1	7		10	1		3	17		
6148–17		1		5			2	1	11	4
6148–21		2		3					2	1
6148–23		1	7	10					2	9
5145–4		2		4				3	4	1
5145–7		6	1	5					9	4
5145–15		4		11		1		5	6	
6148–12				15						17
5145–5				7						11
5145–10			6	1				1	5	
5145–11		3					1	1		

IMMUNE × SUSCEPTIBLE

In Ottawa 770B × Pale Blue and Ottawa 770B × C. I. 479, the not immune segregates varied from class 6 to class 10. Since, as already pointed out, these five classes probably represent only fluctuations due to environment, they have been combined in the genetical analysis of these crosses into a susceptible group. The data on these two crosses when inoculated with the collection of rust are summarized in table 16.

TABLE 16.—*Reaction of parents, and of F_1 and F_3 families of Ottawa 770B × Pale Blue and Ottawa 770B × C. I. 479 to collection of rust in greenhouse*

Parent or generation		Plants or lines showing indicated reaction		
		Immune	Segregating	Susceptible
Ottawa 770B	plants	33		
Pale Blue	do			38
F_1	do	5		
F_3	lines	11	33	10
Ottawa 770B	plants	38		
C. I. 479	do			30
F_3	lines	13	34	17

In Ottawa 770B × Pale Blue, the F_1 was immune like Ottawa 770B. The fit of the F_3 lines to a 1 : 2 : 1 ratio was good, P lying between 0.30 and 0.50. Total χ^2 for fit to a 3 : 1 ratio within the segregating F_3 lines was 33.788 with 33 degrees of freedom. The normal deviate of 0.16 ± 1 was clearly not statistically significant. Likewise, in Ottawa 770B × C. I. 479, the fit of the F_3 lines to the theoretical 1 : 2 : 1 ratio was good, P lying between 0.50 and 0.70. Total χ^2 for fit to a 3 : 1 ratio within the segregating F_3 lines was 26.618 and the normal deviate was −0.889 ± 1. Thus the deviation from the expected ratio was again within the errors of random sampling. These results strongly support the hypothesis of a single dominant factor determining the immunity of Ottawa 770B. The relation of the reaction of the F_3 lines of Ottawa 770B × Pale Blue when inoculated with the collection to their reaction with form 4 is shown in table 17. The agreement of reaction in this cross is complete. A

TABLE 17.—*Correlation table showing the reaction of F_3 lines of Ottawa 770B × Pale Blue and Ottawa 770B × C. I. 479 to collection of rust in greenhouse and to form 4*

OTTAWA 770B × PALE BLUE

Reaction to form 4	Reaction to collection			
	Immune	Segregating	Susceptible	Total
	Number	*Number*	*Number*	*Number*
Immune	11			11
Segregating		33		33
Susceptible			10	10
Total	11	33	10	54

770B × C. I. 479

Immune	12	1		13
Segregating	1	33		34
Susceptible			17	17
Total	13	34	17	64

similar comparison for Ottawa 770B × C. I. 479 is also shown in table 17. In this cross the agreement is again complete except for two lines. These two exceptions have probably resulted from the chance failure to recover recessive segregates among the progeny of heterozygous F_2 plants. It seems probable that, in these crosses, the factor for immunity from the collection of rust likewise conditions immunity from form 4.

The data on the reaction to the collection of rust or the parents and F_2 generation of C. I. 479 × Bolley Golden are presented in table 18

TABLE 18.—*Reaction of parents and F_2 generation of C. I. 479 × Bolley Golden to collection of rust in greenhouse*

Parent or genaration	Plants showing indicated reaction					
	0	1	2	7	9	10
	Number	*Number*	*Number*	*Number*	*Number*	*Number*
Bolley Golden	65					
C. I. 479				20	35	5
F_2	246	38	27	13	3	8

Combining classes 1 and 2 into a resistant group and classes 7, 9, and 10 into a susceptible group, the F_2 ratio was 246 immune : 65 resistant : 24 susceptible. χ^2 for fit of this ratio to an expected ratio of 12:3:1 gave a value of P between 0.70 and 0.80. The results in the 55 F_3 lines are summarized in table 19.

TABLE 19.—*F_3 lines of C. I. 479 × Bolley Golden, grouped according to their breeding behavior when tested with a collection of rust in greenhouse, and the expected ratio on the basis of two factor pairs, one for immunity and one for resistance*

Item	Lines showing indicated reaction [1]						
	I	IR, S	I, R	I, S	R	R, S	S
	Number	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*
Observed	12	7	15	10	5	4	2
Calculated	13.75	13.75	6.875	6.875	3.4375	6.875	3.4375

[1] See footnote 1, table 5.

Combining the last three classes because of the small numbers, χ^2 for goodness of fit was 15.109, a value in excess of χ^2 for the 1-percent point. The greatest deviation of observed from calculated occurs in the F_3 lines segregating for immune, resistant, and susceptible and in those segregating for immune and resistant. In the lines segregating for immune, resistant, and susceptible, on the assumption of two pairs of factors, only one-sixteenth of the plants in the line should be susceptible. Since the average number of plants per F_3 line was only 10.02, susceptible segregates would frequently not be obtained among the progeny of F_2 plants heterozygous for both pairs of factors, in which case the line would be incorrectly classed as segregating for immune and resistant. Since the numbers are too small to permit the accurate differentiation of these two classes, it seems logical to combine them in the analysis. χ^2 for comparing the observed and calculated ratios after combining these two classes, and also combining the last three classes in table 19, gave a value of P between 0.50 and 0.70. These results indicate that the immune strain of Bolley Golden carries a dominant factor conditioning immunity and a dominant factor for resistance to the collection of rust, the factor for immunity being epistatic to the factor for resistance.

TABLE 20.—*Correlation table showing the reaction [1] of F_3 lines of C.I. 479 × Bolley Golden to collection of rust in greenhouse and to form 4*

Reaction to form 4	Reaction to collection							
	I	I, R, S	I, R	I, S	R	R, S	S	Total
	Number	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*
I	10		3		1			14
I, R, S		7		1				8
I, R	1		8	1				10
I, S	1		1	6				8
R					4			4
R, S						4		4
S							2	2
Total	12	7	12	8	5	4	2	50

[1] See footnote 1, table 5.

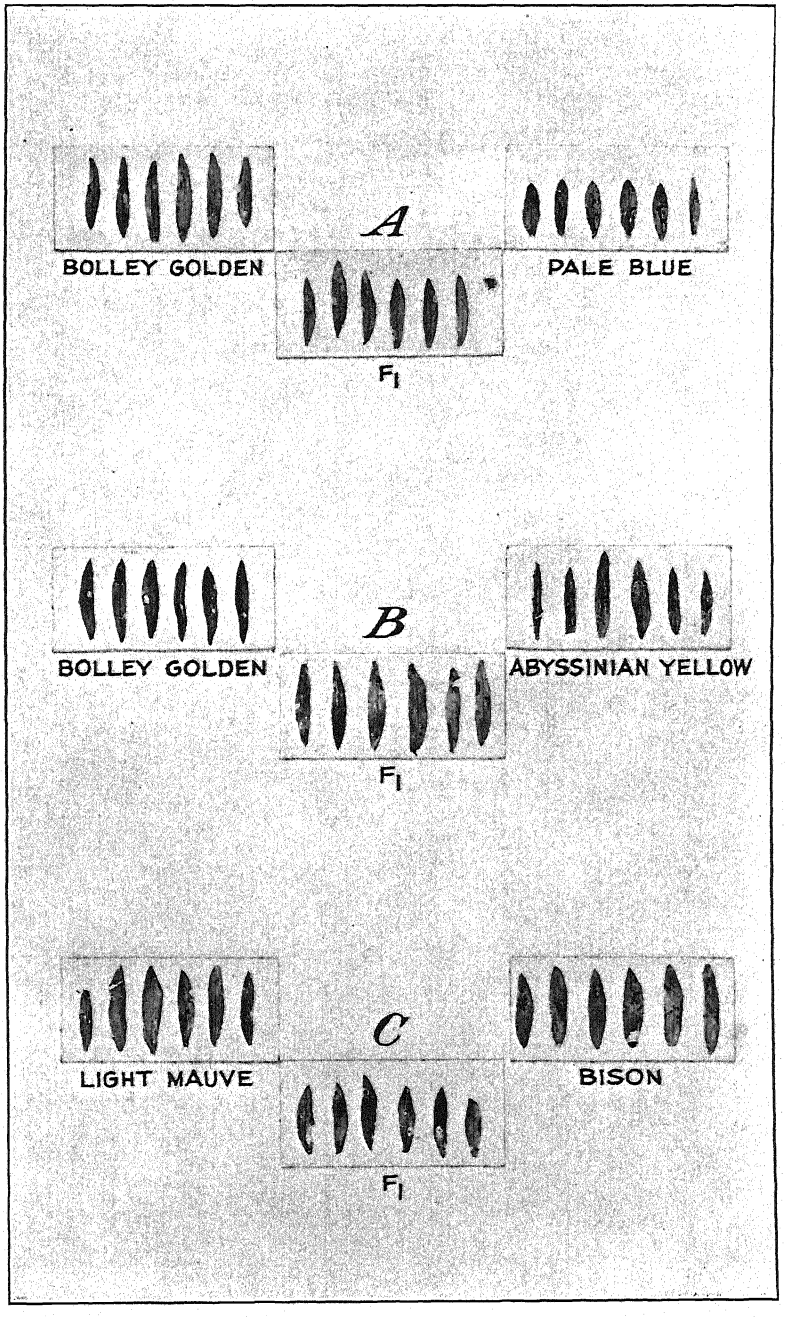
Seed of 50 of the F_3 lines of this cross was available also for testing with form 4. The relation of their reaction to form 4 with their reaction to the collection is shown in table 20. Although several deviations from an exact agreement occur, these differences could have resulted from a failure to recover recessive segregates in some cases owing to the small number of plants per line.

Near Immune × Resistant

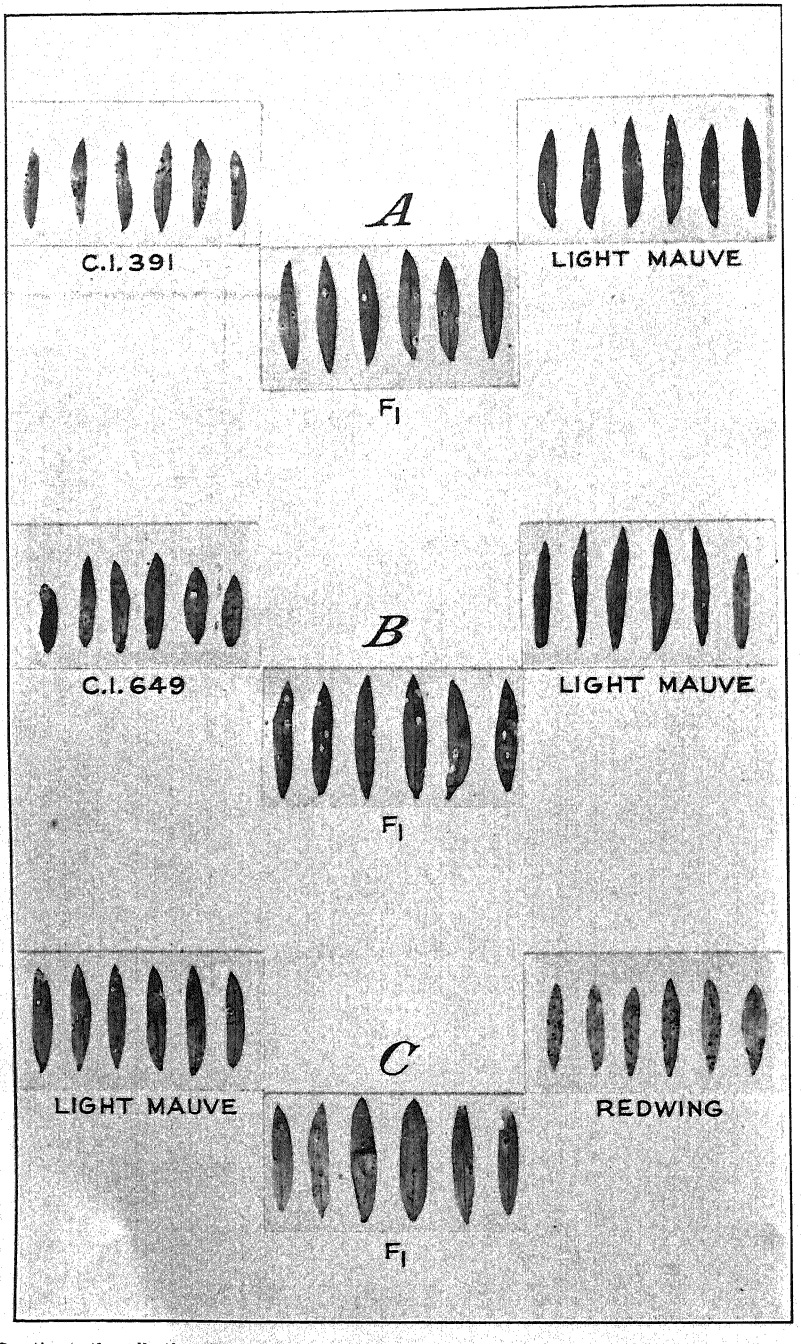
Thirty-four F_3 lines of C. I. 416-3×C. I. 712, averaging 11.6 plants per line, were inoculated with the collection of rust. Because of the overlapping of the near-immune and resistant reaction, it was impossible to distinguish near-immune from resistant segregates. Consequently, the genetic factors differentiating near immunity and resistance could not be determined in this cross. In addition to near-immune and resistant segregates, 3 of the 34 F_3 lines each contained a single semiresistant plant and 1 line contained a susceptible segregate when inoculated with the collection. When plants of the F_3 lines were inoculated with form 4, six lines contained semiresistant and

Table 21.—*Reaction of parents, and of F_1 and F_3 generations of C. I. 712 × (Long × E) to collection of rust in greenhouse and to form 4*

Parent or generation	Plants in rust class indicated when inoculated with—											
	Collection						Form 4					
	0	1	2	3	7	8	0	1	2	3	5	7
	No.	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*
C. I. 712	14						10	7				
Long × E		12	5				1	12	1			
F_1	4	1					1	1				
C. I. 712	9	12					10	4				
Long × E	5	12	3				14	1				
F_3 families:												
6142-4	3	6	7	1			1	12	3	2		
6142-6	1	2	10	4				3	12	1		
6142-8	1	3	7	3				12	5	1		
6142-9	1	1	4	1				2	6	1	6	2
6142-2	2		8	4			2		8			
6142-5		1	2	2				1	7	3		
6142-10		6	6	3			1	15	3			
6142-7	1	1	9	2		2		1	13	2		
6142-3	1	2	4	4	1			6	5	1	1	
6142-1		2	5	1		1	1	8	3	1		
5139-2	14						10	4	3			
5139-4	18						17					
5139-6	14						12	2				
5139-8	16						14					
5139-10	12						9	2				
5139-15	14						16	1				
5139-17	15						14	3				
5139-3	11	1					10	1				
5139-5	13	2					14					
5139-7	3	3						16				
5139-9	13	3					10	3				
5139-11	15	1					13					
5139-14	12	1					4	2				
5139-16	11	2					1	6				
5139-19	3	2						9				
5139-23	10	1					6	1				
5139-36	6	1					1					
5139-27	12	4					12	2				
5139-28	8	4					8	3				
5139-25	12	1	2				4	2				
5139-24		6	2					4	1			



Reaction to the collection of rust of parents and F1 plants: *A*, Bolley Golden × Pale Blue; *B*, Bolley Golden × Abyssinian Yellow; *C*, Light Mauve × Bison.



Reaction to the collection of rust of parents and F_1 plants: *A*, C. I. 391 × Light Mauve; *B*, C. I. 649 × Light Mauve; *C*, Light Mauve × Redwing.

two lines susceptible segregates. These semiresistant and susceptible segregates may have resulted from the absence of allelism of factors conditioning near immunity and resistance in the two parents or from the segregation of minor modifying factors. Insufficient data were available in this cross for selecting between these alternatives.

The results obtained in C. I. 712 × (Long × E) are given in table 21.

One striking feature of the reaction of the F_3 lines when inoculated with the collection was observed. In the progeny of F_2 family 6142 the plants were predominantly class 1, 2, and 3 with a few segregates also in class 0. In addition, 3 lines out of the 10 showed susceptible segregates. On the other hand, progeny of F_2 family 5139 were predominantly class 0, 14 of the 21 F_3 families also having segregates in class 1 and 2. This sharp difference between F_2 families indicates that the two F_1 plants were genetically different, probably as a result of heterozygosity for factors for rust reaction of one or both of the parental lines. A similar situation obtained in the material tested with form 4.

Near Immune × Semiresistant

One cross of near immune × semiresistant, i. e., Light Mauve × Redwing, was studied. When inoculated early in the fall, some plants of Redwing were placed in classes 4 and 5 and some were classed as susceptible. However, the cross Light Mauve × Redwing was inoculated on November 23 when the light intensity and duration were much reduced, and at this time Redwing gave uniformly a susceptible reaction, as is seen in table 22.

Table 22.—*Reaction of parents and of F_1 and F_2 generations of Light Mauve × Redwing to a collection of rust in greenhouse*

Parent or generation	Plants showing indicated reaction					
	0	1	2	3	5	
	Number	*Number*	*Number*	*Number*	*Number*	*Number*
Redwing						16
Light Mauve		3	15			
F_1			2	2		
Redwing						18
Light Mauve	17	3				
F_2	113	20	1	4	11	32

In the additional crosses made in 1934, a resistant plant of Light Mauve was used. The four F_1 plants were classed as two class 2 and two class 3 (pl. 3, *C*). Thus, the resistance of Light Mauve was nearly completely dominant to the susceptibility of Redwing.

Since the Light Mauve plants which descended from the original parent of the F_2 and F_3 generations occurred in both class 0 and class 1, it seemed logical in the analysis of the segregating populations to group the class 0 and class 1 segregates. In addition, the results with the F_1 plants indicate that class 3 was not greatly different genetically from class 2 but instead may have represented a fluctuation due to environment. It is interesting to note that the class 1, 2, and 3 plants in the F_2 generation made up 18.1 percent of the near-immune group (classes 0, 1, 2, and 3), whereas 15 percent of the Light Mauve

plants were classified as class 1. Combining the class 5 and susceptible plants, the ratio in F_2 was 138 near immune:43 semiresistant and susceptible plants. χ^2 for fit of this ratio to a 3:1 gave a value of P between 0.50 and 0.70. This indicates that a single major factor pair differentiates Light Mauve and Redwing as regards reaction to the collection of rust. Twenty-six F_3 lines, averaging 7.63 plants per line, were studied and a ratio of 11 near immune:7 segregating:8 semiresistant and susceptible F_3 lines was obtained. χ^2 for fit to a 1:2:1 ratio gave a value of P between 0.02 and 0.05, such a deviation being expected only 2 to 5 times out of 100 trials due to chance alone. However, with the small number of plants per F_3 line, it is probable that some lines were incorrectly classified, particularly those classed as near immune because of failure to recover the expected recessive segregates. Insufficient seed of the F_3 lines of this cross was available for testing with form 4.

Resistant × Semiresistant

The data on rust reaction of the parents, and of the F_1 and F_2 generations of Light Mauve × Bison are presented in table 23.

Table 23.—*Reaction of parents and F_1 and F_2 generations of Light Mauve × Bison to collection of rust in greenhouse and to form 4*

Parent or generation	Plants showing indicated reaction when inoculated with—										
	Collection					Form 4					
	1	2	3	5	7	0	1	2	3	5	7
	Number	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*
Light Mauve	1	6				4	24	6			
Bison			2	8	9				7	26	1
F_1	1	3	1			1	1				
F_2	13	90	22	15	6	9	89	62	6	11	

In the material inoculated with the collection, the five F_1 plants were placed in classes 1, 2, and 3 (pl. 2, *C*), the resistance of Light Mauve being almost completely dominant over the semiresistance of Bison. The overlapping of Bison and the F_1 in class 3 was a source of difficulty in analyzing the data, particularly in the F_2. On the basis of parental reaction, 10.5 percent of the plants of the genotype of Bison should occur in class 3. If the 21 plants in classes 5 and 7 are used as the basis for calculating the F_2 plants with the same genotype as Bison, 2.5 of the class 3 plants should be of that genotype. Combining the remaining class 3 plants with the resistant group, since the data on F_1 indicates that part of the heterozygous plants will also be in class 3, the ratio was 122.5:23.5. χ^2 for fit of this ratio to a 3:1 gave a value of P slightly above 0.01. Twenty-nine F_3 lines were inoculated with the collection and a ratio of 9 resistant : 12 segregating : 8 semiresistant and susceptible lines was obtained. Five of the eight lines classed as semiresistant and susceptible contained class 3 plants, but, since class 3 plants also occur in Bison, this seemed legitimate. When this ratio was compared with a 1:2:1 by means of χ^2, P was between

0.50 and 0.70. The results in F_3 indicate that, although the deviation from the expected was rather large in F_2, Light Mauve is probably differentiated from Bison by a single major factor conditioning resistance to the collection of rust.

Since classes 0, 1, and 2 plants occurred in Light Mauve when tested to form 4, these were combined in analyzing the F_2 data on reaction to form 4. Likewise, classes 3 and 5 both were found in Bison and these two classes were also combined in F_2. This gave a ratio of 160:17. χ^2 for fit of this ratio to 15:1 gave a value of P between 0.05 and 0.10, suggesting that the resistance of Light Mauve to form 4 may be conditioned by duplicate factors. Unfortunately, insufficient seed of the F_3 lines was available for a satisfactory test of their reaction to form 4. Therefore it was impossible to draw definite conclusions regarding the inheritance of resistance to form 4 in this cross.

The reaction of the parents, F_1 and F_2 generations of C.I. 391 × Light Mauve to the collection, is given in table 24.

TABLE 24.—*Reaction of parents and of F_1 and F_2 generations of C. I. 391 × Light Mauve to collection of rust in greenhouse*

Parent or generation	Plants showing indicated reaction				
	1	2	3	5	7
	Number	*Number*	*Number*	*Number*	*Number*
C. I. 391				14	16
Light Mauve	8	26			
F_1		4		1	
F_2	9	67	11	14	10

All plants of Light Mauve were placed in classes 1 and 2, while the plants of C. I. 391 were placed in classes 5 and 7. Of the five F_1 plants, four were placed in class 2 (pl. 3, *A*) and one was placed in class 5. Since C. I. 391 was used as the female parent, it is probable that this single class 5 plant was selfed C. I. 391 rather than a hybrid. In the F_2, combining class 3 with the resistant group and considering classes 5 and 7 together, a ratio of 87 resistant : 24 semiresistant and susceptible plants is obtained. The fit to a 3:1 ratio is good, P lying between 0.30 and 0.50, thus supporting the evidence of a single dominant factor conditioning the resistance of Light Mauve to the collection.

RESISTANT × SUSCEPTIBLE

Two crosses of resistant × susceptible, involving Bolley Golden with Pale Blue and with Abyssinian Yellow were studied. All plants of Bolley Golden inoculated as checks with these crosses were placed in classes 1 and 2, whereas all check plants of Pale Blue and Abyssinian Yellow were in class 7. The hybrid plants, both in F_2 and F_3, occurred in classes 1, 2, 6, and 7. In the analysis of the data, classes 1 and 2 were combined into a resistant group, while classes 6 and 7 were considered as susceptible. This seemed logical not only on the basis of parental reaction, but also because of the sharp difference between the segregates of the two different groups. The data on the reaction of these two crosses to the collection of rust are summarized in table 25.

TABLE 25.—*Reaction of parents and of F_1, F_2, and F_3 generations of Bolley Golden × Pale Blue and Bolley Golden × Abyssinian Yellow to collection of rust in greenhouse*

Parent or generation		Plants or lines showing indicated reaction		
		Resistant	Segregating	Susceptible
Bolley Golden	plants	76		
Pale Blue	do			70
F_1	do	4		
F_2	do	225		73
F_3	lines	7	16	9
Bolley Golden	plants	51		
Abyssinian Yellow	do			34
F_1	do	3		
F_2	do	109		36
F_3	lines	12	8	13

In both crosses, the F_1 plants were resistant (pl. 2, *A* and *B*). In the F_2 generation of Bolley Golden × Pale Blue, χ^2 for fit to a 3:1 ratio gave a value of P between 0.80 and 0.90. Comparing the ratio of F_3 lines to 1:2:1 by means of χ^2 gave, in this case, a value of P between 0.80 and 0.90. The total χ^2 for fit to a 3:1 ratio within the segregating F_3 lines was 5.351 and P was in excess of 0.99. Such a good fit is expected less than once in 100 trials. However, the number of plants per F_3 line was only 10.3 and such a low χ^2 value may have resulted, in part at least, from the small numbers.

In the F_2 generation of Bolley Golden × Abyssinian Yellow, χ^2 for fit to a 3:1 ratio gave a P value between 0.95 and 0.98. When the ratio of F_3 lines was compared with a 1:2:1 ratio, P lies between 0.01 and 0.02. However, the average number of plants per F_3 line was only 10.4. Therefore, susceptible segregates would not always be recovered in the progeny of heterozygous F_2 plants, in which case the lines would be classed as resistant. Total χ^2 for fit to a 3:1 ratio within the segregating F_3 lines gave a P value between 0.98 and 0.99. Also in this case the low χ^2 value may have resulted from the small number of plants in the F_3 lines. The preponderance of evidence in these two crosses is in support of the assumption of a single dominant factor conditioning the resistance of Bolley Golden to the collection, a hypothesis suggested by the results obtained in Ottawa 770B × Bolley Golden. Material of these two crosses was likewise tested with form 4, and the data on the reaction of parents and F_1 and F_2 generations are given in table 26.

TABLE 26.—*Reaction of parents, and of F_1 and F_2 generations of Bolley Golden × Pale Blue and Bolley Golden × Abyssinian Yellow to form 4*

Parent or generation	Plants showing indicated reaction			
	0	1	2	7
	Number	*Number*	*Number*	*Number*
Bolley Golden	20			
Pale Blue				18
F_1	4			
Bolley Golden		14	4	
Pale Blue				18
F_2		42	103	39
Bolley Golden		34	3	
Abyssinian Yellow				38
F_2		64	62	53

The 20 plants of Bolley Golden that were progeny of the plant used in the cross with Pale Blue in the summer of 1934 were all class 0 when inoculated with form 4. Likewise, the four F_1 plants were class 0. The 18 plants of Bolley Golden which were progeny of the original parent of the cross from which the F_2 material of Bolley Golden × Pale Blue descended were placed in classes 1 and 2. All plants of Pale Blue were in class 7, i. e., susceptible. Combining the class 1 and 2 segregates in F_2 into a resistant group, the ratio was 145:39. χ^2 for fit of this ratio to 3:1 gave a P value between 0.20 and 0.30. In Bolley Golden × Abyssinian Yellow, combining classes 1 and 2, a ratio of 126:53 was obtained. χ^2 for fit to a 3:1 ratio gave a P value between 0.10 and 0.20.

The association of the reaction of the F_3 lines of Bolley Golden × Pale Blue to the collection with their reaction to form 4 is shown in table 27. It can be seen from these data that the agreement is almost complete. In the one line classed as resistant to the collection and segregating with form 4, only nine plants were tested to the collection. Likewise, in the two lines classed as segregating with the collection and resistant to form 4, only six and four plants, respectively, were tested with form 4. A similar situation is shown in table 27 for the F_3 lines of Bolley Golden × Abyssinian Yellow.

TABLE 27.—*Correlation table showing the reaction of F_3 lines of Bolley Golden × Pale Blue and Bolley Golden × Abyssinian Yellow to collection of rust in greenhouse and to form 4*

BOLLEY GOLDEN × PALE BLUE

Reaction to form 4	Reaction to collection			
	Resistant	Segregating	Susceptible	Total
	Number	*Number*	*Number*	*Number*
Resistant	6	2		8
Segregating	1	10		11
Susceptible			9	9
Total	7	12	9	28

BOLLEY GOLDEN × ABYSSINIAN YELLOW

Resistant	5			5
Segregating	1	6		7
Susceptible			9	9
Total	6	6	9	21

In the one line classified as resistant to the collection and segregating with form 4, only eight plants were tested to the collection. These results indicate that the factor which conditions the resistance of Bolley Golden to the collection also determines its resistance to form 4.

SEMIRESISTANT × SUSCEPTIBLE

The data on reaction of parents and F_1 and F_3 generations of C. I. 479 × Bison and Pale Blue × Bison to the collection, are summarized in table 28.

TABLE 28.—*Reaction* [1] *of parents and of F_1 and F_3 generations of C. I. 479 × Bison and Pale Blue × Bison to collection of rust in greenhouse*

Parent or generation		Number of plants or lines showing indicated reaction		
		SR	SR, S	S
C. I. 479	plants			33
Bison	do	26		11
F_1	do	4		
F_3	lines	7	20	4
Pale Blue	plants			40
Bison	do			33
F_1	do			5
F_3	lines		27	37

[1] See footnote 1, table 5.

In C. I. 479 × Bison, all of the plants of C. I. 479 were classified as susceptible. The plants of Bison varied from semiresistant to susceptible and the four F_1 plants were placed in class 5, indicating dominance of semiresistance in this case. Of the 31 F_3 lines studied, 7 contained only semiresistant (class 4 and 5) plants, 20 contained both semiresistant and susceptible segregates, and 4 were classed as susceptible. One of the four susceptible lines also contained a single class 5 plant which might have resulted from genetical segregation or which might have been a natural hybrid. In view of the variability of Bison from semiresistant to susceptible, an attempt to give an exact factorial explanation of the results obtained in this cross does not seem expedient. When these F_3 lines were inoculated with form 4, class 3 segregates occurred in 10 of the lines. Likewise, 4 of the 34 plants of Bison were placed in class 3. Again no factorial explanation of the results could be given.

All of the plants of Bison, Pale Blue, and the F_1 of the cross Pale Blue × Bison were classed as susceptible. This behavior of Bison is unique so far as these studies are concerned, although Flor (*3*) reported Bison as susceptible in his studies of greenhouse reaction. The variability of the reaction of Bison from semiresistant to susceptible in other inoculations in this experiment indicates that its reaction may be readily influenced by the environment. Therefore, it is perhaps not surprising to find conditions under which all plants of Bison are susceptible. In the 64 F_3 lines of this cross, 27 contained both class 4 and susceptible plants, the remaining 37 lines being susceptible. Again, in this cross, it does not seem possible to give a factorial explanation of the results.

CROSSES INVOLVING C. I. 649

Since the analysis of the reaction of the check plants of C. I. 649 showed that this variety was quite variable in its rust reaction, it seemed desirable to discuss the crosses in which it was involved together.

The reaction of the parents and F_2 generation of Ottawa 770B × C. I. 649 to a collection of rust is shown in table 29.

The plants of C. I. 649 ranged in reaction from class 1 to class 5. Combining these four classes in the F_2 generation, the F_2 plants are found to occur in the ratio of 426:134:31. χ^2 for fit of this ratio to a

12:3:1 gave a value of P between 0.02 and 0.05. Thus a deviation as great or greater than this would be expected by chance only 2 to 5 times in 100 trials.

TABLE 29.—*Reaction of parents and F_2 generation of Ottawa 770B × C. I. 649 to collection of rust in greenhouse*

Parent or generation	Plants showing indicated reaction					
	0	1	2	3	5	7
	Number	*Number*	*Number*	*Number*	*Number*	*Number*
Ottawa 770B	75					
C. I. 649		8	5	35	20	
F_2	426	27	6	51	50	31

In the crosses involving C. I. 649 with Light Mauve and with the three semiresistant varieties, Bison, Redwing, and C. I. 391, considerable killing of the hybrid material as well as the plants of C. I. 649 occurred in the incubators when an incubation period of 48 hours was used. All material of these crosses was replanted and an incubation period of 24 hours was used. Almost no injury occurred with this shorter period in the incubators. However, this necessity of replanting reduced the supply of seed so that insufficient seed was available for testing with form 4. The reaction to the collection of rust of parents, F_1, F_2, and F_3 generations of these crosses is summarized in table 30. No factorial explanation can be given for the results in these crosses, although it seems probable that multiple factors were involved in determining the type of reaction of C. I. 649.

TABLE 30.—*Reaction* [1] *of parents, and of F_1, F_2, and F_3 generations of crosses involving C. I. 649 to collection of rust in greenhouse*

Parent or generation	Number of plants or lines showing indicated reaction						
	R	R,SR,S	R,SR	R,S	SR	SR,S	S
Light Mauve—plants	29						
F_1—do	5						
F_3—lines	17	5	4	1			
C. I. 649—plants	17				2		
Bison—do					25		1
F_1—do	1				4		
F_2—do	50				57		2
F_3—lines	5	9	13	2	1	1	
C. I. 649—plants	9				8		
Redwing—do							22
F_1—do					3		1
F_2—do	17				52		30
F_3—lines		12	11		2	6	
C. I. 391—plants					11		19
C. I. 649—do	34						
F_1—do					4		
F_2—do	43				137		7

[1] See footnote 1, table 5.

CORRELATION OF FIELD AND GREENHOUSE REACTION

For the plant breeder, a knowledge of the extent of correlation of field reaction with the rust reaction of young plants in the greenhouse is of considerable importance. The reaction of the parents

both under field conditions and in the greenhouse has already been discussed. Two varieties, Long × E and C. I. 416–3, which showed a near-immune reaction in the greenhouse, and C. I. 712, resistant under greenhouse conditions, were immune in the field. The reaction of the other varieties in the greenhouse was comparable to their field reaction, except C. I. 649, which was somewhat less resistant in the greenhouse than in the field.

The critical test of whether the same genetic factors govern field and greenhouse reaction is afforded by a study of hybrid material. Such information was available in only two crosses in this study. Progenies of 54 of the 55 F_2 plants of Ottawa 770B × Pale Blue, which were studied in the field, were inoculated in the greenhouse both with the collection and with form 4. Of the 44 F_2 plants that were immune in the field, 11 had only immune progeny in the greenhouse, while the progeny of 33 plants segregated for immune and susceptible. The 10 F_2 plants that were susceptible in the field had only susceptible progeny in the greenhouse. The rust reaction in the field of 99 F_3 lines of Ottawa 770B × Redwing has been discussed. Seed was available for testing in the greenhouse of 92 of these lines and they were inoculated with form 4. The relation between the field reaction and the reaction to form 4 of the F_3 lines is shown in table 31.

TABLE 31.—*Correlation table showing the reaction of F_3 lines of Ottawa 770B × Redwing to rust in the field and to form 4 in the greenhouse*

Reaction to form 4	Lines showing indicated field reaction			
	Immune	Segregating	Rusted	Total
	Number	*Number*	*Number*	*Number*
Immune	27	2		29
Segregating		42		42
Rusted			21	21
Total	27	44	21	92

The results obtained in these two crosses indicate that the same genetic factor conditions the immunity of Ottawa 770B in the greenhouse and in the field.

DISCUSSION

A summary of the reaction to the collection of rust in intercrosses of the immune, near-immune, and resistant varieties and the suggested genotypes of these varieties are presented in table 32.

The results obtained in all of these crosses except those involving Long × E can be explained by assuming factors in two different allelic series, in which L and M are duplicate factors conditioning immunity from the collection. l^n and m^n are duplicate factors conditioning near immunity, l^n being allelic to L and m^n allelic to M. l^r and m^r are duplicate factors conditioning resistance to the collection, l^r being allelic to L and l^n and m^r being allelic to M and m^n. On this basis, susceptible segregates or varieties would carry the recessive allels in each series and would be of the genotype $ll\ mm$.

TABLE 32.—*Summary of the reaction to the collection of rust in intercrosses of immune, near-immune, and resistant varieties and the suggested genotypes of these varieties*

Variety and genotype	Ottawa 770B *LL mm*	C. I. 438 *LL* $m^r m^r$	Newland *ll MM*	C. I. 416–3 *ll* $m^n m^n$	Long × E $l^n l^n$ $m^n m^n$
C. I. 438 *LL* $m^r m^r$.	Immune				
Newland *ll MM*.	Immune and susceptible.	Immune and resistant.			
C. I. 416–3 *ll* $m^n m^n$.	Immune, near immune, and susceptible.	Immune	Immune and near immune.		
Long × E $l^n l^n$ $m^n m^n$.	Immune and near immune.	Immune, near immune, and a few susceptible.	do	Immune and near immune.	
C. I. 712 $l^r l^r$ *mm*.	Immune, resistant, and a few semiresistant.	Immune and resistant.	Immune, resistant, and susceptible.	Near immune, resistant, and a few susceptible.	Near immune, resistant, and susceptible.
Bolley Golden	Immune, resistant, and susceptible.				
Light Mauve	do				

The proportion of susceptible segregates in the cross of Ottawa 770B × Newland indicated that the immunity of these two varieties was, in each case, conditioned by a single dominant factor, the factors in the two varieties not being allelic. Studies of seven other crosses of Ottawa 770B with resistant, semiresistant, and susceptible varieties confirmed the assumption of a single dominant factor for immunity in Ottawa 770B. Likewise, the results in C. I. 712 × Newland substantiated the assumption of a single dominant factor conditioning the immunity of Newland. On this basis, the genotype of Ottawa 770B could be written *LL mm* and that of Newland *ll MM*. The occurrence of susceptible segregates in C. I. 416–3 × Ottawa 770B indicated that C. I. 416–3 did not carry a factor for near immunity allelic to *L* while the absence of such segregates in C. I. 416–3 × Newland suggested that C. I. 416–3 carried the factor m^n for near immunity which was allelic to the *M* factor carried by Newland.

Susceptible segregates were obtained in C. I. 712 × Newland, suggesting that C. I. 712 did not carry a factor for resistance allelic to the *M* factor of Newland. In Ottawa 770B × C. I. 712, a few semiresistant segregates obtained. These could have resulted from the segregation of minor modifying factors with C. I. 712 carrying the l^r factor for resistance allelic to the factor of Ottawa 770B. On this assumption, susceptible segregates would be expected in C. I. 712 × C. I. 416–3. Actually, a few semiresistant and susceptible segregates were found in this cross, but fewer than would be expected on the hypothesis. It is possible that these segregates were likewise conditioned by modifying factors and that these two varieties carried a factor for resistance not allelic to either *L* or *M*. However, the evidence is not conclusive.

Results in the crosses of C. I. 438 with Ottawa 770B and Newland suggest that C. I. 438 carried the same factor that conditions the immunity of Ottawa 770B and a factor for resistance, m^r, which was allelic to the *M* factor carried by Newland. Thus the genotype of C. I. 438 would be *LL* $m^r m^r$. This was further substantiated by the results in crosses of C. I. 438 with C. I. 712 and C. I. 416–3. No

semiresistant or susceptible segregates obtained in either of these crosses. The behavior in crosses involving Long × E was not entirely consistent. No susceptible segregates were obtained either in crosses of (Long × E) × Newland or Ottawa 770B × (Long × E), suggesting that Long × E carried factors allelic to both the *L* and *M* factors. On this basis, no susceptible segregates would be expected in crosses of Long × E with C. I. 438 or C. I. 712, whereas susceptible segregates were obtained in both crosses. Results in both of these crosses indicated that one or both parents used in making the crosses were heterozygous, a fact which might have accounted for the susceptible segregates.

Crosses of the resistant strain of Light Mauve with two semiresistant varieties, Bison and C. I. 391, indicated that a single dominant major factor determined the resistance of Light Mauve. Likewise, the results in crosses of the resistant strain of Bolley Golden with the two susceptible varieties, Pale Blue and Abyssinian Yellow, were explained by the assumption of a single dominant factor conditioning the resistance of Bolley Golden. In crosses of these two varieties with Ottawa 770B, about one-sixteenth of the F_2 segregates were semiresistant or susceptible, indicating that the factors for resistance carried by these two varieties were not allelic to the *L* factor conditioning immunity in Ottawa 770B. No crosses were available either for determining whether these two resistant varieties, Light Mauve and Bolley Golden, carry the same factor for resistance or for determining the relationship with the *M* factor of these factors for resistance.

The immune strain of Bolley Golden was found to carry a single dominant factor for immunity and another dominant factor conditioning resistance which was hypostatic to the factor for immunity. The results in the cross of the near-immune strain of Light Mauve with Redwing suggested a single factor difference between these two varieties as regards reaction to the collection of rust. No crosses were available for studying the relation of the factors carried by these two strains with the factors for immunity, near immunity, or resistance contributed by the other varieties studied.

A definite factorial explanation could not be given for the inheritance of the C. I. 649 type of reaction. The results suggested that multiple factors might be involved. Likewise it was impossible to place the inheritance of the semiresistant type of reaction on a definite factorial basis.

SUMMARY

The nature and interaction of genes conditioning different types of reaction to rust and the relationship of genes conditioning the same type of rust reaction in different varieties was studied with physiologic form 4 and a collection of rust. Thirty-seven crosses involving 17 strains and varieties of flax were used in these studies.

In the field studies, Ottawa 770B, C. I. 438, Newland, Long × E, C. I. 416–3, C. I. 712, and one strain of Bolley Golden were found to be immune from rust. One strain of Bolley Golden and the varieties Light Mauve and C. I. 649 were resistant, Bison and Redwing were semiresistant, and C. I. 391, Pale Blue, and Abyssinian Yellow were moderately susceptible. The immunity of Ottawa 770B in the field was conditioned by a single dominant factor.

In the greenhouse studies with the collection of rust, Ottawa 770B, Newland, C. I. 438, and one strain of Bolley Golden were immune. Long × E, C. I. 416–3, and one strain of Light Mauve were near immune. C. I. 712 and strains of Bolley Golden and Light Mauve were resistant. Bison, Redwing, and C. I. 391 were semiresistant and Pale Blue and Abyssinian Yellow were susceptible. C. I. 649 gave a mixed reaction varying from resistant to semiresistant.

Immunity was dominant to near immunity, resistance, and susceptibility, and resistance was dominant to semiresistance and susceptibility in the crosses used in this study.

The reaction to the collection of rust of crosses involving Ottawa 770B, Newland, C. I. 438, C. I. 416–3, and C. I. 712 was explained by assuming factors in two different allelic series, *L* and *M*. *L* and *M* are duplicate factors conditioning immunity. l^n and m^n condition near immunity, l^n being allelic to *L* and m^n allelic to *M*. l^r and m^r condition resistance to the collection, l^r being allelic to *L* and l^n and m^r allelic to *M* and m^n. On the basis of this hypothesis, the genotype of Ottawa 770B is *LL mm;* Newland, *ll MM;* C. I. 438, *LL* m^rm^r; C. I. 416–3, *ll* m^nm^n, and C. I. 712, l^rl^r *mm*.

Results in crosses involving Long × E indicated that it probably carried the l^n and m^n factors. The occurrence of susceptible segregates in C. I. 438 × (Long × E) and C. I. 712 × (Long × E) may have resulted from heterozygosity of the parental material.

A single major factor apparently conditioned resistance to the collection both in the resistant strain of Light Mauve and in Bolley Golden. Crosses with Ottawa 770B indicated that neither of these varieties carried a factor for resistance allelic with *L*. No crosses were available for determining the relationship of the factor or factors in these varieties with the *M* series of allels.

The immune strain of Bolley Golden carried two factors, conditioning immunity and resistance, respectively, to the collection. A single major dominant factor conditioned the near immunity of the strain of Light Mauve to the collection. No data were available for determining the relationship of factors in these two strains with *L* or *M* series of allels.

No factorial explanation could be given for the inheritance of the C. I. 649 type of reaction or of the semiresistant reaction.

Reaction of the parents to form 4 was similar to their reaction to the collection. Bison and C. I. 649 seemed somewhat more resistant and Redwing and C. I. 391 somewhat more susceptible to form 4. This difference may have been conditioned by environmental factors.

In general the reaction of hybrids to form 4 was similar to their reaction to the collection. Results indicated that the same factors conditioned the immunity of Ottawa 770B and Newland from the collection and from form 4. The factors determining immunity and resistance to the collection in crosses involving the immune strain of Bolley Golden likewise conditioned immunity and resistance, respectively, to form 4. In hybrids involving the resistant strain of Bolley Golden, the same factor conditioned resistance both to the collection and to form 4. There was evidence that the m^r factor of C. I. 438, which conditioned resistance to the collection, determined immunity from form 4. Two duplicate factors appeared to be conditioning the resistance of Light Mauve to form 4. However, this evidence was not conclusive.

The factor conditioning the immunity of Ottawa 770B in the field also determined its immunity in the greenhouse both when inoculated with the collection and with form 4.

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TWIG LESIONS AS A SOURCE OF EARLY SPRING INFECTION BY THE PEAR SCAB ORGANISM[1]

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INTRODUCTION

Pear scab (*Venturia pyrina* Aderh.) has been known to be present in the Hood River Valley of Oregon for the last 20 years but has become of commercial concern only since 1932. During that year two pear (*Pyrus communis* L.) orchards produced as much as 80 percent of scabby fruit, and in 1934 plantings at a distance of nearly 2 miles from the original infection centers showed some scab. The disease has increased also in parts of southern and western Oregon and western Washington.

In the course of experiments for scab control in the Hood River Valley, conducted by the writers, several new facts concerning the life cycle of the pear scab organism in relation to control measures were established. The early dispersal of conidia from overwintering scab lesions on twigs, the effect of spray materials in relation to this phase of the disease, and the comparative importance of conidia and ascospores in initiating primary infections are especially worthy of consideration in this connection.

TWIG INFECTION

HISTORICAL REVIEW

Frequent reference has been made to shoot infection on trees since Aderhold (*1*)[3] first described pear scab as occurring on young branches. English writers generally agree that conidia produced from this type of carry-over are almost entirely responsible for primary spring infections, and that ascospore discharge from leaf material is of only minor importance (*5*, *14*). The more outstanding contributions are reviewed by Marsh (*14*). In Australasia, workers generally have found that ascospores appear to be more important in initiating primary infections than conidia produced by the fungus overwintering on the young shoot growth. Data substantiating this view are found in publications by Cunningham (*3*), Curtis (*4*), Hearman (*10*), and Pittman (*16*) and in additional references cited in these papers. Dowson (*6*), however, emphasizes the importance of twig lesions as a source of infection at the time when sprayings are practically over.

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[3] Reference is made by number (italic) to Literature Cited, p. 681.

Very few published data have appeared in the United States, especially in recent years, on the twig phase of pear scab, or on entirely effective control measures. Duggar (*7*) has given a general survey of earlier work in this country, and Heald (*9*) has supplemented it. Smith (*17*) found that under California conditions primary infections were due to spores liberated from scab pustules on wood growth of the past season. His observations and experimental work were substantiated by the results of commercial field tests for the control of the disease. Thomas (*18*), however, concluded that ascospores are the principal source of primary infections in California since a search in commercial orchards in 1929 revealed that the great majority of the current season's lesions had been circumscribed by a cork layer before the end of the growing season.

Differences of opinion appear to exist also among investigators of the Pacific Northwest. Fisher and Newcomer (*8*, *p. 7*) state:

> In the treatment of pear scab it is important to dispose of all possible sources of infection, and to this end twig cankers, if present, should be removed in pruning and the infected wood burned. Some disposition should also be made of fallen leaves which harbor the fungus over winter, and which are the most important source of early spring infection.

Although not ignoring the function of this ascospore material, Jackson (*11*) pointed out that the disease was more difficult to control in Oregon where twig infections were present, and that several seasons might be required to rid the orchard of this source of infection.

Other than a brief statement by Marsh (*14*) on twig scab control, the writers' preliminary report (*13*) appears to constitute the only record of the effect of sprays on this phase of the disease. Pathologists have tended to base control recommendations on results obtained with the closely related apple scab, but such recommendations have sometimes been found of doubtful application to pears, especially in relation to twig lesions as overwintering sources of infection.

VARIETAL SUSCEPTIBILITY

Approximately half the pear acreage of the Hood River Valley is planted to Anjou, a variety very susceptible to twig attack. Easter Beurre (planted mainly as a pollinator), Flemish Beauty, and Forelle are also very susceptible, but they constitute a minor portion of the plantings in this locality.

Fruit of the Bartlett, the second leading variety, is often slightly affected, especially when interplanted among other heavily infected trees, but twig infections are extremely rare. Bosc is intermediate between Anjou and Bartlett in respect to both fruit and twig attack.

The fact that various degrees of susceptibility have been assigned to these same varieties in other regions indicates that environmental responses or specialized strains of the organism may exert an influence on infection.

In a small planting bordering the Hood River Valley, Bartlett twigs have been found severely scabbed. The greater precipitation and humidity in this section appear to be the factors that allow the parasite to attack this variety so severely.

CYCLE OF INFECTION

New growth of susceptible twigs may be infected at any time during the growing season, but infection occurs more commonly in the Hood

River Valley during spring months when rainfall is frequent. During spring and summer months, new lesions appear merely as small blister-like cushions, often with a prominent lenticular spot at their centers, or on certain varieties as shallow spore-producing stromata. Occasionally the host forms a corky layer beneath the cushion or stroma and partially sloughs it off during the current season. This type is

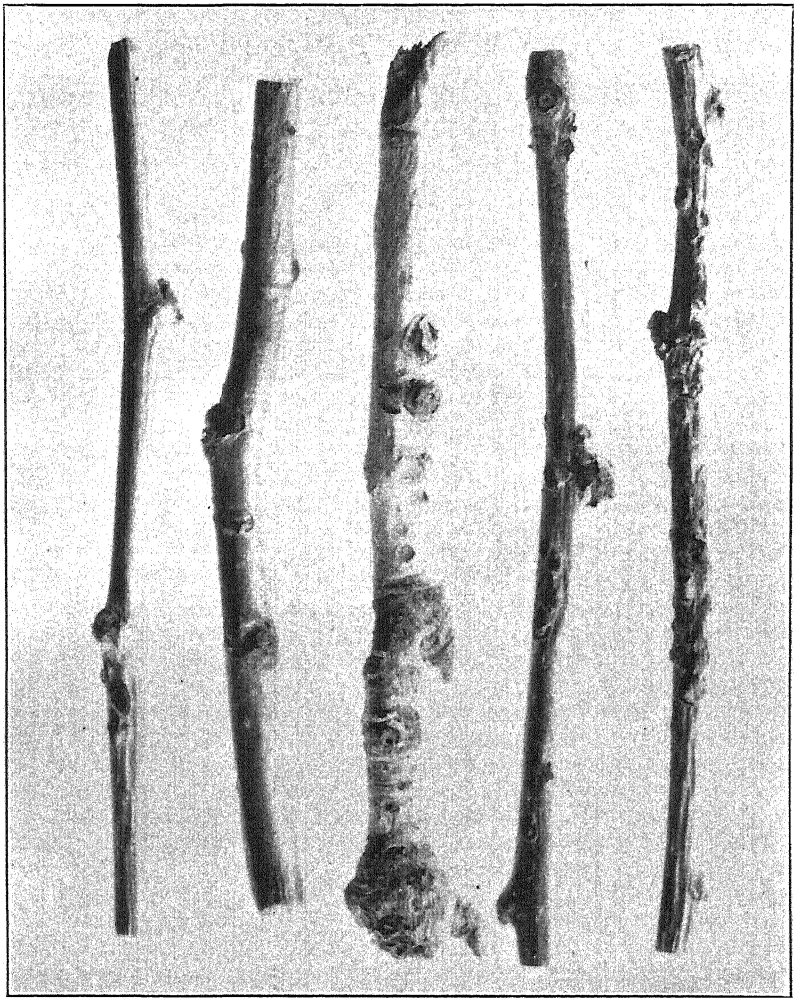


FIGURE 1.—Pear scab on Anjou twigs. Middle twig shows depressions remaining on 2-year-old wood after the scab spots have been sloughed off. From material collected during May.

more easily seen, appearing as a small cankerlike injury. After the primary establishment of the fungus it usually remains more or less inactive until winter, when the trees become dormant. Active enlargement of the fungus fruiting structure then occurs, and by early spring conidial formation has started. Dissemination of these conidia takes place during rainy periods throughout the season or until the

pustules become sloughed off by renewed tree activity. (For a detailed account of the histological features of this cycle, see Marsh's article (*14*), which is substantiated by the writers' observations.) These twig infections (fig. 1) are generally sloughed off during the growing season or before the tree again becomes dormant, although occasionally a few remain partially attached and contain viable conidia the next season. In this case the fungus penetrates the host barrier and may form a new pustule unless a second abscission layer is successful in arresting its growth. Oftentimes 4- and 5-year-old wood still shows evidence of previous infections in the form of circular depressions. Only a short period of activity of these twig pustules is necessary to cause primary spring infections, a fact which Thomas (*18*) appears to have overlooked.

EXPERIMENTAL METHODS

Three orchards in the Hood River Valley were examined in 1934 and two of them again in 1935 to discover at what time primary infection occurred and which spore form was involved. The first orchard was severely infected in both years, and previous studies showed that humidity had reached 100 percent on all but four nights during the preceding summer. Scab had been present for several years, and conditions for its development were ideal, since 100 percent of the unsprayed fruit was affected. In the second orchard, which was believed to represent more closely the general run of orchards in which scab had obtained a foothold, less than 50 percent of the unsprayed fruit had been scabby the previous year. The Oregon State Experiment Station's orchard at Hood River, where scab had never been present, was used as a check plot.

Spore traps were made by tying together, back to back, two slides with outer surfaces coated with petrolatum. Three traps per tree were tied in a vertical free-hanging position at heights of 2, 8, and 16 feet above ground in a representative tree of each orchard. One square inch of each slide was examined under the microscope each week or after periods of heavy rain, for the purpose of recording spore catches.

Ascospore discharge records from overwintered leaves, brought to the laboratory and exposed to natural conditions, were also kept. Five leaves bearing perithecia were placed in a shallow box on a natural orchard soil covering. Ordinary glass slides were then placed directly over each leaf on narrow wood supports to keep them from being in contact with the leaf surface. This procedure is essentially the one described by one of the writers (*2*) in a report of apple scab studies. Discharged spores readily stuck to the glass surface. These slides were examined at the same periods as the spore traps hanging in the orchard. Because of the numbers involved, however, the average number of ascospores caught per square millimeter was used to bring these values in line with other charted data.

SEASONAL DEVELOPMENT AND SPORE LOAD IN RELATION TO SCAB

Records of the weather and of the volume of spores in relation to the prevalence of pear scab in the experiments of 1934 and 1935 are shown graphically in figures 2 and 3. Weather data in the charts are given

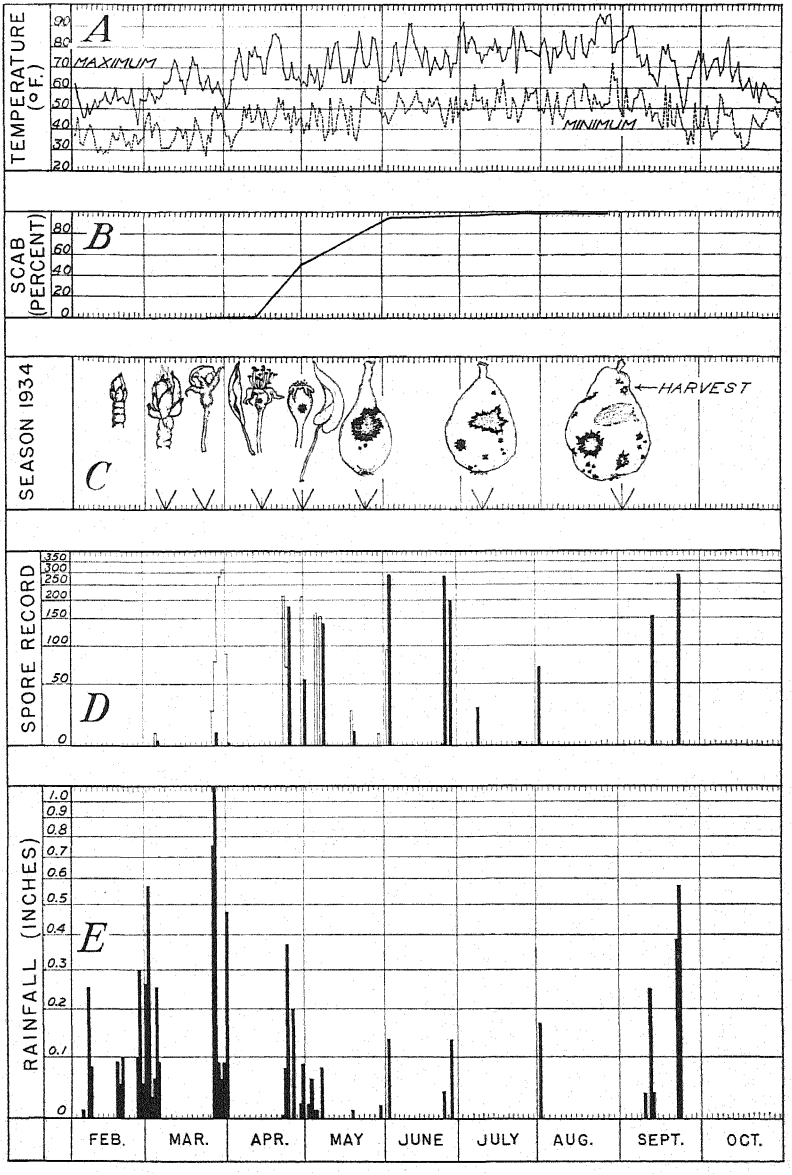


FIGURE 2.—Weather and spore records in relation to pear scab, 1934: *A*, Maximum and minimum temperatures; *B*, scab infection on fruit of unsprayed trees; *C*, stages of development and infection of buds, blossoms, and fruit; *D*, record of spores; light lines coming to a point represent number of ascospores per square millimeter from captive leaves; black bars represent number of conidia per square inch (6¼ square centimeters) caught in orchard; *E*, record of rainfall.

for the station at Hood River, since records are unavailable for the other orchards in which experiments were conducted.

SPORE RECORDS FOR 1934

It has been pointed out by previous workers that ascospore discharge occurs only during rainy periods and that conidia of the fungus are

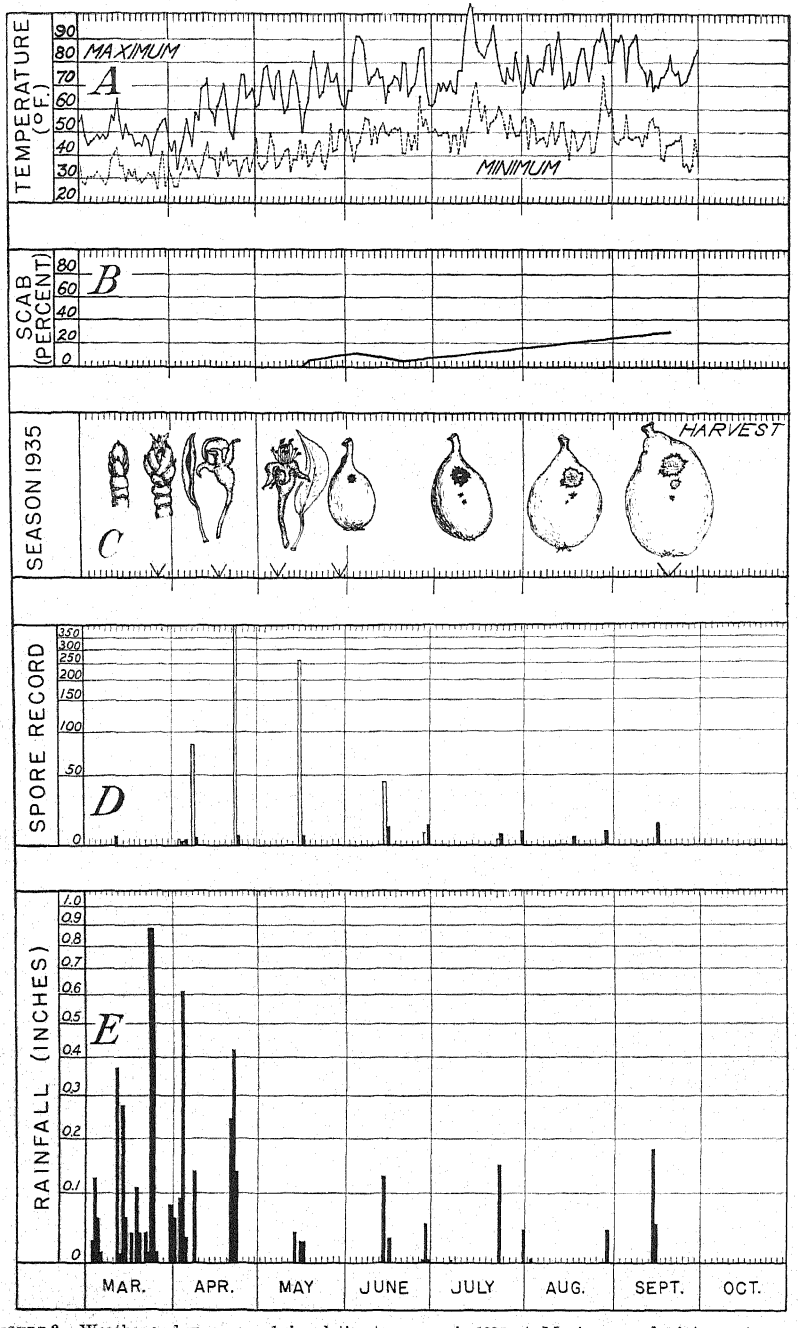


FIGURE 3.—Weather and spore records in relation to pear scab, 1935: *A*, Maximum and minimum temperatures; *B*, scab infection on unsprayed trees; *C*, stages of development and infection of buds, blossoms, and fruit; *D*, record of spores; light lines coming to a point represent number of ascospores per square millimeter from captive leaves; black bars represent number of conidia per square inch (6¼ square centimeters) caught in orchard; *E*, record of rainfall.

readily dislodged by moisture but not by wind alone. Figure 2, *D* and *E*, shows this relation for ascospores from captive leaves and for conidia caught on slides hanging in heavily infected trees during the 1934 season at Hood River. Although ascospores were caught in large numbers on slides placed directly above overwintered leaves, only three were captured during the entire season on the traps hanging in the trees. These three were recorded April 30, June 4, and June 25. Conidial catches in the trees, however, were comparatively large. A local shower fell in the orchard July 9, which explains why conidia were caught at that time. Although total rainfall was slightly higher in this orchard than at the Hood River station orchard, except for July 9, the periods of distribution were the same.

The first catch of conidia was made during the rainy period starting February 26. It is doubtful whether infection could have occurred at that time, since the buds were not open (fig. 2, *C*). Unusually high temperatures during the early spring months, however (fig. 2, *A*), favored rapid growth, so that by March 26, the start of the next rainy period, which yielded the first conidial catch of any consequence, the young leaves and flower buds were exposed.

An examination was made each day to determine the incubation period of the organism. The first scab symptom, a slight greenish fuzz, was found on young fruits April 14, 19 days after the beginning of the second rainy period, which, as previously stated, coincided with the first conidial catch of any consequence. Leaf infections were not found until a few days after the fruit-infection stage and were never numerous except on unsprayed trees. Figure 2, *B*, illustrates the importance of twig lesions as primary infection sources on Anjou pears. It can be seen that even though the spore record shows a comparatively small catch of conidia, half of the fruits on unsprayed trees became infected from these sources.

These primary spots on fruits and other susceptible tissues produced new conidial spore material in such amounts that, with the following rainy period, practically all fruits became infected. Conidial spore catches on the traps in the orchard, of course, increased accordingly. Counts of infected fruits during several stages in their development showed that new spots appeared on susceptible varieties following each rainy period of sufficient duration to allow the fungus to become established. With certain varieties, such as Bosc, the fruits had developed such resistance by the time they were one-third grown that new infections were extremely rare. Scab spots already present gradually died out, and little evidence of scab was seen at harvest unless the fruit had become misshapen. Bartlett pears, which have never become excessively scabby in the Hood River Valley, exhibited a high degree of resistance throughout their cycle of development when associated with severely scabbed Anjous. It appears at this time that, even with an abundant source of spore material, Bosc pears require protection only during early spring, whereas control can be obtained on Anjous only by complete protection throughout the season.

Figure 4 records catches of conidia from the moderately infected orchard. Direct comparisons of unsprayed trees of the heavily and the moderately infected orchards cannot be made, since the owner of the latter did not wish to leave the fruits unprotected. By observing pears in the poorly sprayed treetops, however, it was roughly

estimated that approximately half as much scab developed in this orchard as on the unsprayed fruit of the heavily infected orchard.

No scab spores were caught on slides in the orchard which had not contained scab, and which was some distance from any infected orchard.

It was assumed that if the original spore load from infected wood could be reduced the percentage of scabby fruit showing primary lesions and the chances for reinfections to occur would be lessened accordingly. Figure 5 represents results from two trees that had received no spray in 1933 and that were used to test this assumption. One was left unsprayed; the other received three lime-sulphur sprays in 1934. It can readily be seen that infection was reduced, but reduction of fruit scab approximated only 50 percent because of poor spraying. Figure 4 contrasts the same unsprayed tree with one in a moderately infected orchard receiving three early sprays of a partially effective fungicide. Conidial catches were greatly reduced, but practically half the fruits became scabby because of infections resulting when spray coverages were largely dissipated.

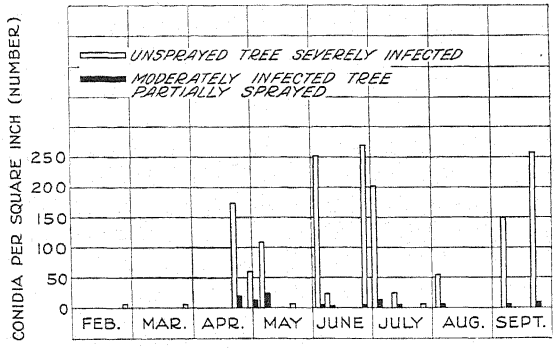


FIGURE 4.—Relation of orchard infection to conidial catch, 1934. (Periodic amounts of rainfall for figs. 4, 5, and 6 may be determined by referring to fig. 2.)

Figure 6 compares conidial numbers caught on slide traps suspended on unsprayed trees at three elevations. The lower and middle heights yielded similar conidial ratios throughout the season, whereas the highest trap was noticeably most free from conidia at each period. Since conidia are washed downward by rains, they naturally are found in greater numbers at the lower tree levels; but it should be borne in mind that a few infected fruits or twigs in the top of the tree are more favorably situated to scatter conidia to healthy fruits than are those situated lower down. Since so few ascospores were caught in the trees, they were considered of minor importance in causing the heavier infection nearer the ground, where, because they are produced in leaves on the ground, they might be of importance if present in larger numbers.

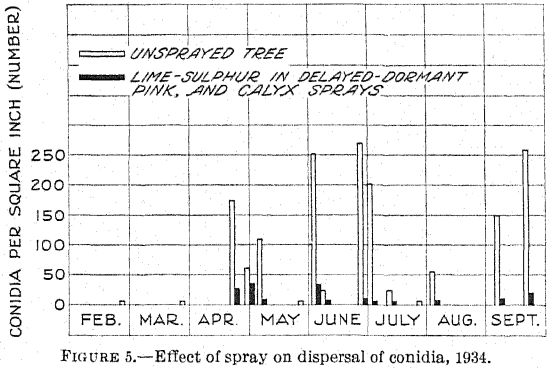


FIGURE 5.—Effect of spray on dispersal of conidia, 1934.

SPORE RECORDS FOR 1935

The year 1935 threatened to be an exceptionally bad scab year, since new wood was heavily infected and the fungus had become established in practically all sections of the valley. Scab, however, did not develop to any considerable extent, for the following reasons:

(1) Before the trees became dormant the previous fall, 80 percent of the twig scab infections became sloughed off or inactivated. (Note the number of inactive scab pustules on twigs of the unsprayed plot in table 2.)

(2) In early spring there was only one rain sufficient to cause scab infection. Where spray recommendations were followed, there was nearly complete protection. (See fig. 3.)

(3) Few new infections appeared following the light rains in May, June, and July, principally because infective material was scarce. Inoculum for subsequent infections was much decreased by the dropping off of infected fruits at the time of the "June drop." Practically all fruits showing pedicel infections were lost at that time.

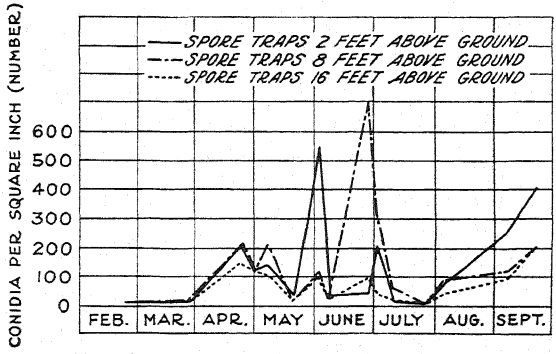


FIGURE 6.—Relation of height of traps to conidial catch, 1934.

Prospects for a severe scab season were somewhat dissipated after the discovery that a large percentage of the twig lesions had become inactivated. For previous experiments to be substantiated it would have to follow that a correspondingly smaller number of conidia would be available for primary infections. That this was actually true can be determined from figure 3, *D*. It should be noted that actual conidial catches early in the 1935 season were slightly less than in the previous year, but the potential sources would have been far greater (see tables 1 and 2) if the wood lesions had overwintered in an active state. An increase in conidial material from secondary infections was small, owing to weather factors previously mentioned. As a result, fruit was much cleaner at harvest than during the previous season when secondary conidial material became increasingly larger up to midseason. Numerous primary infections and favorable moisture conditions later were responsible for the rapid increase of scab in 1934.

Three ascospores constituted the total catch for the 1934 season on nine traps hanging on trees in the orchard. Nine were captured during 1935 in comparable trials from March 1 to the last of May, five of which were recorded on April 21. The primary fruit infections appeared on May 15.

Neither conidia nor ascospores were captured in the check-plot orchard where scab had never been present.

The results of the writers' experience in orchard spraying on a commercial scale during past years correspond very closely with those reported above. When a thorough and properly timed spray was

applied before primary infections had become established and was followed later with a reasonable spray program, satisfactory control of scab was realized at harvest. If, however, the first spray was poorly applied or improperly timed, so that a few primary infections occurred in spite of careful and frequent subsequent sprayings there was considerable scabby fruit at harvest.

CONTROL OF TWIG SCAB

The data given above and observations made during past years indicate that primary spring infections resulted largely from conidia derived from active lesions on scabbed wood of the previous season's growth, and that these conidia were being dispersed even before susceptible tissues were exposed. Ascospore discharge was also recorded from the overwintered leaves, but whether this would occur every year remains to be determined. It is possible, however, that if early sprays are timed by ascospore discharge in orchards where active twig scab occurs, primary infections could already result from twig conidia before the regular spray schedule begins. It has been repeatedly pointed out for both pear and apple scab that the amount of early infection usually influences the number of scabby fruits present at harvest. Any practice that eliminates or checks dispersal of these twig conidia, then, is of paramount importance in control.

EFFECT OF SPRAYS

Data on the effect of fungicides in controlling twig scab are extremely meager. In the orchard used for scab-control experiments, twig infections were exceedingly common and evidence of their existence could be seen on wood several years old when spraying tests were started. Some variation in the total number of shoot pustules per tree occurred, but the orchard could be classified as severely infected and favorable for scab development.

A spray schedule consisting of lime-sulphur 1–12 applied in the delayed-dormant stage and of other materials applied in the pink and calyx stages was employed during the 1933 and 1934 seasons. One additional cover spray was applied in 1933 and two in 1934. Twig counts in the spring following such treatments yielded evidence that sprays measurably control the twig lesions (tables 1 and 2). It should

TABLE 1.—*Effect of sprays on pear twig scab the year following their application, 1933–34*

1933 spray treatment (Delayed-dormant lime-sulphur 1-12 plus 3 later applications)	Scab pustules, 1934			Total twigs infected
	On 50 twigs	Average, per twig	Maximum on any twig	
	Number	*Number*	*Number*	*Percent*
None (west check)	364	7.3	29	90
None (east check)	230	4.6	30	86
Copper oxide-lime-bentonite (2-4-2-50)	30	.6	10	16
Copper phosphate-lime-bentonite (2-4-2-50)	16	.3	3	20
Wettable sulphur no. 1 (10-100) in pink and calyx stage plus bordeaux 3-6-50 in 2 later covers	74	1.5	25	34
Wettable sulphur no. 1 (10-100)	44	.9	7	42
Wettable sulphur no. 2 (10-100)	62	1.2	17	42
Flotation sulphur (6-100)	25	.5	5	28
Lime-sulphur (½-50) and wettable sulphur no. 1 (10-100)	11	.22	4	12
Lime-sulphur (1-50) in pink, wettable sulphur no. 1 (10-100) later	20	.4	12	10
Lime-sulphur (1-50) in pink and calyx, then wettable sulphur no. 1 (10-100)	3	.06	2	4
Lime-sulphur (1-50) in pink, calyx, first cover, then wettable sulphur no. 1 (10-100)	8	.16	2	14

TABLE 2.—*Effect of sprays on pear twig scab the year following their application, 1934–35*

1934 spray treatment (Delayed-dormant lime-sulphur 1-12 plus 4 later applications)	Orchard no.	Scab pustules (1935)						Total twigs infected
		On 50 twigs		Average per twig		Maximum on 1 twig		
		Active [1]	Inactive [2]	Active	Inactive	Active	Inactive	
		Number	*Number*	*Number*	*Number*	*Number*	*Number*	*Percent*
Check, no spray	1	41	429	0.82	8.58	7	30	88
	2	122	486	2.44	9.72	9	32	100
Zinc sulphate-lime (4-4-50) plus lime-sulphur (1-100) in covers	1	16	21	.32	.42	3	12	18
	2	33	33	.66	.66	4	4	60
Copper silicate (3-100); thereafter 1½-100	1	1	7	.02	.14	1	2	12
	2	6	6	.12	.12	1	2	22
Bordeaux-lime-bentonite (1-2-1-50)	1	1	0	.02	0	1	0	2
	2	1	3	.02	.06	1	1	8
Copper phosphate-lime-bentonite (2-4-2-50)	1	0	2	0	.04	0	2	2
	2	2	1	.04	.02	1	1	6
Copper oxide-lime-bentonite (2-4-2-50)	1	5	3	.10	.06	1	2	12
	2	14	29	.28	.58	2	4	48
Flotation sulphur-bentonite (3-3-50) (1 pound lime)	1	3	4	.06	.08	1	1	14
	2	1	8	.02	.16	1	2	14
Lime-sulphur (1-100) pink; wettable sulphur no. 1 (10-100) thereafter	1	6	1	.12	.02	1	1	14
	2	5	5	.10	.10	1	1	18
Lime-sulphur lime-bentonite (½-1-2-50)	1	1	1	.02	.02	1	1	4
	2	1	1	.02	.02	1	1	4
Wettable sulphur no. 1 (8-100)	1	6	31	.12	.62	1	10	26
	2	16	44	.32	.88	2	4	62
Bordeaux (1-50) plus oil (½-100) in pink; (1-100) thereafter	1	1	3	.02	.06	1	2	4
	2	1	0	.02	0	1	0	2

[1] Active scab pustules.
[2] Sloughed or inactive pustules.

especially be noted that the wettable sulphur types of materials were much less effective in this control than the more caustic sulphurs or chemicals giving a longer coverage. The same general trend was apparent in fruit scab control. Where mild sprays were used during the early stages of growth it was very noticeable that control of scab on both fruit and twigs was comparatively poor, whereas their substitution at later periods showed less difference.

Figure 5 shows that conidial catches were greatly reduced on sprayed trees. To determine the exact cause of this decrease in conidial numbers early in the season, twigs with active scab pustules were brought into the laboratory and sprayed with certain fungicides. Table 3 gives the results of this test. Lime-sulphur completely inactivated such pustules by an actual "burning out" action. The entire spore-bearing surface was killed and the stroma soon became flat and crusty and did not revive when placed in a moist chamber. In contrast to this, although other fungicides tested caused a considerable decrease in active conidial material where the fungicide was in actual contact with the spore-bearing parts, penetration under the epidermal covering of the pustule was much inferior to that of lime-sulphur. Pustules sprayed with these chemicals, moreover, partially revived in moist chambers and produced some conidia. The same results were observed under field conditions.

TABLE 3.—*Effect of sprays on conidia in pustules on excised twigs*

Spray treatment	Spore germination	Location of spores in pustule	Spray treatment	Spore germination	Location of spores in pustule
Apr. 29, 1935			1934		
	Percent			*Percent*	
Check, no spray	76	Various points.	Wettable sulphur (10-100)	70	Edge.
Lime-sulphur (1-15)	0	Edge.		3.2	Center.
	0	Center.			
Bordeaux mixture (4-4-50)	45	Edge.			
	2.5	Center.			

These results indicate that lime-sulphur applied after twig lesions are open and active should largely eliminate this source of infection. The majority of pustules at Hood River during past years opened before blossom buds became exposed. A small percentage, however, opened during or after this period. It was known from previous experience that lime-sulphur could not be used with safety on Anjou pears in this locality after the bud scales had dropped. For this reason it was believed that best results would be obtained in scab control by delaying the initial lime-sulphur application until the bud scales were just ready to drop. Where it is possible to use this caustic spray on other varieties at a later date, successful control should be possible in one season. Pustules that opened late or those that remained active during the summer contained viable spores at harvest and formed dangerous sources for fall fruit infections. Water sprouts remained susceptible to infection late in the season and required protection where scab was a problem.

A secondary influence of spray materials was apparent on sprayed trees. It appeared that during the spring the host was able to circumscribe scab lesions on twigs more quickly on sprayed trees than on those receiving no treatment. Whether this action was due to a partial killing of the parasite or to a favorable effect on the host was not determined.

RESIDUAL EFFECT OF SPRAYS

The severely infected orchard used for part of these experiments offered an extreme test of the cumulative value of proper and well-timed spray applications. When a part of this orchard was taken over by the writers for spray tests at the end of the 1933 season, 68.3 percent of the commercially but improperly sprayed fruit and 100 percent of the unsprayed pears were scabby. In the 1934 tests the applications of four sprays of various materials, following the delayed-dormant lime-sulphur spray, reduced scab to as low as 12 percent when the stronger fungicides were used. This plot was given back to the grower for the 1935 season because of certain conditions complicating records on fruit russet. However, comparison of this plot with the remainder of the orchard in the less severe scab year of 1935 showed that an average infection of 1.8 percent occurred in the previously well-sprayed section (including all materials tested), while 28 percent of scab was present in the part sprayed entirely by the grower during both years. These results, in conjunction with data given in tables 1 and 2, seem to warrant the conclusion that this

residual effect of spray materials was almost entirely due to the reduction of twig infections, since few ascospores were caught in the trees during either year.

Effect of Dormant Sprays

The possibility of killing the parasite on twigs in its inactive and overwintering form was tried by means of penetrating spray materials developed by Keitt (*12*) for dormant use. During this period the fungus is not exposed, and a penetrating material would be required to reach the vulnerable tissue. Although killing of the fungus occurred, a certain percentage of the pustules continued to become active in the spring, even on trees showing distinct and severe spray injury in the form of partially killed limbs. It is quite possible, however, that if further improved or used in different combinations such materials may find a useful place in the spray schedule for pear scab control. This point needs further investigation.

Effect of Cultural Practices

Certain horticultural practices may influence twig scab infections. The cutting out of infected shoot material has been generally recommended as an aid in controlling pear scab. This practice may be helpful in eliminating some of the original spore load, but even then it is usually not complete enough to be entirely effective without additional protection to new growth. Pruning stimulates the production of new wood, which must be protected to prevent reinfection. Since watersprout types of growth are especially susceptible to shoot infection, however, their removal from the central parts of the tree should be practiced. Pruning so as to produce the most open type of tree without sacrificing bearing surfaces appears to be most desirable from the standpoint of tree vigor, thorough spraying, and partial elimination of spore material.

Unpruned trees and trees very low in vigor have been found to show less infection, since they fail to produce much new and succulent growth.

DISCUSSION

The results obtained on the relation of twig lesions to primary scab infections on pear trees are in accord with work done in England, and indicate that these lesions furnish the bulk of material for early infection under certain environmental conditions. Observations and the spraying of commercial orchards in Oregon during the last 4 years have further substantiated these data. A few twig pustules are easily overlooked in an orchard, but they may be surprisingly persistent in dissemination of the parasite and when favorable environmental conditions are at hand may cause serious epidemics. Where these twig infections are absent it has been relatively easy to control pear scab, even when ascospores from overwintering leaves have been plentiful. The latter, however, should not be ignored, since a certain percentage do find their way to susceptible tissues. Results from widely scattered countries suggest that environmental factors may influence their dissemination. The work of Wiesmann (*19*) and Palmiter (*15*) has shown that specialized strains of pear and apple scab exist, a fact that

gives further importance to this sexual stage in the possible production of new forms.

The long, dry growing season of 1934 was of special interest in its relation to twig attack. Most of the pustules occurred at the bases of the current season's twigs, indicating that infection had taken place soon after they started growth. Twig lesions resulting from infections during the early part of 1934 were sloughed off or became inactive before the trees became dormant, and the majority failed to produce conidia in the spring of 1935. During years with shorter growing seasons and more rainfall, however, the host appeared to be unable to circumscribe incipient infections. As a result, practically all twig lesions remained active and produced conidia the following spring. This may explain the conflicting observations made by Smith (*17*) and Thomas (*18*) in California in different years. It may also explain the occasional and sudden decrease in scab following several seasons in which the disease was difficult to control.

The pronounced effect of spray materials in preventing twig infections and in keeping the old lesions from functioning as infection sources seems conclusive, and spraying appears to offer the most economical and practical means of coping with this phase of the disease. Since the more effective materials can be applied to certain varieties with safety only before the blossom cluster buds become exposed, they should be applied at that time carefully and thoroughly. When properly applied even mild fungicides give considerable protection from reinfection to new twig growth.

SUMMARY

Since 1932 pear scab has become a serious factor in pear production in the Hood River Valley of Oregon. The number of primary infections appeared to correlate closely with the amount of twig infections present. Primary spring infections resulted largely from conidia in overwintering pustules on the previous season's wood rather than from ascospores, and few of the latter were ever trapped in trees. Moreover, conidia were being dispersed before bud tissues were exposed.

Early sprays should be timed by conidial dispersion from twig lesions, where these occur, rather than by ascospore discharge, because infection results from these twig spores before ascospores are matured. Consistent and thorough spraying during the growing season largely prevented twig infections. Early-season sprays were more important in this district for control of twig scab as well as of fruit scab, because more precipitation occurred early in the season and because a certain amount of host resistance became apparent after that time.

Lime-sulphur was effective in "burning out" active twig pustules, but it could not be used on tender-skinned varieties after the young fruit was exposed, without causing injury. This fungicide was found to be dangerous if applied after the bud scales had dropped. Applied in the delayed-dormant stage, lime-sulphur reduced primary spore numbers so that additional sprays gave satisfactory protection against reinfection.

Environmental factors play an important role in natural control.

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PHYSIOLOGIC RACES OF USTILAGO HORDEI[1]

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INTRODUCTION

Covered smut of barley (*Ustilago hordei* (Pers.) Kell. and Sw.) in the United States has long been recognized as an important hazard in the culture of barley (*Hordeum vulgare* L.). In the years from 1918 to 1935 for which records are available,[2] its estimated toll averaged over 2¼ million bushels annually, and twice during these years the estimated annual loss exceeded 5 million bushels. Despite recent notable advances in the control of barley covered smut through seed treatment, as shown by Leukel (*10*),[3] relatively little progress has been made in combating the disease through selecting and breeding barleys having inherent resistance to various physiologic races in the smut species. Until recently, progress in this direction had been almost completely blocked by the lack of an effective and easily applied method of artificial inoculation of the seed for producing high percentages of smutted plants. Despite the general belief of long standing that barley is inoculated, in threshing, by the spread of the spores to the surface of the seed, the artificial blackening of seed with millions of spores usually has failed to result in high percentages of smutted plants. Jensen (*9*) first reported this difficulty nearly 50 years ago. In 1934, the writer (*15*) devised a spore-suspension method of inoculating seed barley in which spores are washed beneath the hulls. The method is similar in principle to Haarring's (*5*) "evacuation" method of inoculating oats with smut, but it employs no vacuum or nutrient solution. The spore-suspension method is effective, fairly easy to apply, and approaches the natural method of inoculation as recently reported (*17*). The way thus was opened for the studies on physiologic races presented herein.

PREVIOUS INVESTIGATIONS

Faris (*3*, *4*) first reported physiologic races in *Ustilago hordei* in 1924. Five pathogenic races were isolated. Rodenhiser (*11*), in 1928, described seven cultural races, and a further test of two of these

[1] Received for publication July 9, 1937; issued December 1937. Investigations conducted in cooperation with the New York (Cornell) and North Carolina Agricultural Experiment Stations. A number of the smut collections and lots of barley seed used in the experiments were collected by the late J. A. Faris, formerly senior pathologist, Division of Cereal Crops and Diseases, Bureau of Plant Industry. The writer also has had access to the records of Faris' experiments on physiologic races of *Ustilago hordei*, conducted at Kearneysville, W. Va., in 1932 and 1933, in cooperation with the West Virginia Agricultural Experiment Station, and at Fargo, N. Dak., in 1933, in cooperation with the North Dakota Agricultural Experiment Station.

[2] UNITED STATES BUREAU OF PLANT INDUSTRY. ESTIMATE OF CROP LOSSES DUE TO PLANT DISEASES. 1917 U. S. Dept. Agr., Bur. Plant Indus. Plant Disease Bull. 2:1-18, 1918. [Mimeographed.]

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[3] Reference is made by number (italic) to Literature Cited, p. 691.

showed differences in their pathogenicity on Lion and Himalaya barleys. Recently Aamodt and Johnston (*2*) found two races in Alberta, Canada. Apparently this report completes the list of available contributions. The fact that there have been relatively few investigations of physiologic races of *U. hordei* doubtless is due largely to the difficulties with seed inoculation, as noted previously.

MATERIALS AND METHODS

The experiments were conducted at Ithaca, N. Y., during the 3-year period 1934–36. Eight pure-line varieties of spring barley were used as differential hosts, namely, Excelsior (C. I.[4] 1248), Gatami (C. I. 575), Hannchen (C. I. 531), Lion (C. I. 923), Nepal (C. I. 595), Odessa (C. I. 934), Pannier (C. I. 1330), and Trebi (C. I. 936). The selection of these varieties was based largely on unpublished studies of the late Dr. J. A. Faris. Faris conducted an experiment at Fargo, N. Dak., in 1933, to determine suitable differential barleys and physiologic races of *Ustilago hordei*. Thirty varieties inoculated with each of 65 smut collections comprised the test. The maximum percentage of smutted heads was 28, in the variety Odessa, which proved to be susceptible to every smut collection. In most of the other varieties the maximum smut infection rarely exceeded 15 percent. Although the data were inadequate to warrant definite conclusions, they were useful to the writer in indicating that certain collections of smut were representative of distinct races and that the varieties named above should make good differential hosts. Faris (*3*, *4*) also had found that Hannchen and Nepal well differentiated two smut races which he numbered "1" and "2."

For differential hosts, the writer chose, so far as possible, from the varieties listed by Harlan and Martini (*6*), those that are outstanding for certain characteristics and representative of some barley-growing region of the world. In the course of study, the following additional species and varieties also were tested for their suitability as differential hosts: Hillsa (C. I. 1604), *Hordeum deficiens* Steud. (C. I. 668–1), *H. intermedium* Koern. and Wern. (C. I. 4377), Lyallpur (C. I. 3403), Club Mariout (C. I. 261), Oderbrucker (C. I. 1529), Summit (C. I. 929), and White Smyrna (C. I. 910). The first four proved too highly resistant to be useful, and the remainder failed to further or better differentiate any of the smut races thus far isolated with the varieties selected. Twenty-eight winter varieties or selections were also tested to determine their possible value in differentiating physiologic races of covered smut.

The 8 selected varieties, during the 3-year test period, were inoculated with each of 200 collections of covered smut obtained from 26 States. Preparatory to inoculation, seed of the differential hosts was soaked in a formaldehyde solution (1 part of formaldehyde to 320 parts of water) for 2 hours, washed in running water for one-half hour to remove all traces of formaldehyde, and spread in thin layers until

[4] C. I. refers to accession number of the Division of Cereal Crops and Diseases, formerly Office of Cereal Investigations.

thoroughly dry. Each year five check rows of uninoculated seed of each variety so treated produced smut-free plants. In view of the occurrence of mixed collections of the different barley smuts, the spores of the collections used each year were first examined to determine the character of the epispore and the type of germination on 2-percent potato-dextrose agar. Later, the smutted heads resulting from seed inoculation with each collection were examined in the different varieties. All collections that were suspected or determined to be other than true barley covered smut were eliminated.

In 1934, 1935, and 1936, respectively, 75, 60, and 65 new smut collections were tested. The 1934 collections comprised six races; in 1935 two other races were isolated, but in 1936 no additional races were obtained. Two collections of each of the six races obtained in 1934 were further tested in 1935 and 1936, and two collections of each of the two races obtained in 1935 were again tested in 1936. In these further tests of the isolated races, the inoculum of each race to be used in the succeeding year's test was collected on the same date and subsequently stored in a cool room. Whenever possible, the inoculum was collected from the variety which most clearly differentiated each race to aid in screening out any other races that might have occurred in the original collection. A month or less before seeding, the seed was inoculated by the spore-suspension method previously described (*15*).

The inoculated seed in each year's test was planted at the rate of 6 g to the 5-foot row. The new collections were tested in duplicated systematically distributed rows. In the further tests, in 1935 and 1936, of previously isolated races, triplicated systematically distributed rows were employed. In preparing the inoculum, in inoculating, and in planting the seed, adequate precautions were observed to prevent mixing of spores of different collections. The smut percentages obtained were based on counts of the total number of heads per row in 1934 and 1935 and of 300 heads per row in 1936.

In the analysis of data three infection classes were used, as follows: 0–5 percent infection=resistant class (R); 6–35 percent infection=intermediate class (I); 36 percent infection and above=susceptible class (S).

The classification is arbitrary and the limits of the infection classes have been selected to fit the present data. The purpose of the classification is to present in a convenient way the host reactions in the present study that differentiated the various races of covered smut. As shown in table 1, increase in susceptibility frequently is accompanied by an increase in the variability of infection. The progressive widening in limits of the intermediate and susceptible classes accommodates these variations. In some previous studies of cereal smuts, narrower infection ranges have been considered significant for separating physiologic races. In the present study, however, the variations in different years and between replications in a single year frequently were too great to warrant the use of a classification with more than three classes of infection. In the three seasons in which the tests

were conducted at Ithaca, N. Y., extremes of drought, precipitation, and heat occurred during the growing seasons of 1934, 1935, and 1936, respectively. Under such conditions, the separation of smut races doubtless should be based only on differences in pathogenicity that are fairly wide and reasonably consistent.

In each of the 3 successive years of the experiment, the maximum percentages of smutted heads in the susceptible variety Odessa were 62, 54, and 50, respectively. In Nepal, the maximum percentages were 60, 45, and 58, respectively. The conditions for infection thus appear to have been adequate for the differentiation of physiologic races.

RESULTS

IDENTIFICATION OF PHYSIOLOGIC RACES

The annual and average percentages of smut produced by each of the eight races of *Ustilago hordei*, in 2 or 3 years of the tests, are given in table 1. In each year the two collections of each race produced similar results. Data for only one collection of each race therefore are recorded. As noted previously, some of the smut collections used were collected by Faris. These collections were numbered, but their physiologic race identity was unknown except that one collection was labeled "form 1" and another "form 2." In the present experiments the reaction of these races on the varieties Hannchen and Nepal was similar to that described by Faris (*4*), and Faris' numbers were therefore retained. The varietal reactions which differentiate the races are given in table 2, and this is followed by a key to facilitate the identification of the races. Table 2 and the key show that the eight smut races may be identified with only five of the eight varieties used. However, two of the varieties omitted, Gatami and Trebi, have given good differential reactions with some races and have been useful in confirming their identity. Odessa, also omitted from the key, is needed to perpetuate race 8.

The total number of collections obtained from each of the 26 States that served as sources of the collections, and also the number of races and their relative prevalence in each of these States as indicated by the collections used in these experiments, are given in table 3.

TABLE 1.—*Percentages of smutted heads in 8 varieties of spring barley inoculated with 8 physiologic races of Ustilago hordei, and grown at Ithaca, N. Y., 1934–36*

Race No.	Year tested	Smutted heads in—							
		Excelsior (C. I. 1248)	Gatami (C. I. 575)	Hannchen (C. I. 531)	Lion (C. I. 923)	Nepal (C. I. 595)	Odessa (C. I. 934)	Pannier (C. I. 1330)	Trebi (C. I. 936)
		Percent	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
1	1934	0.0	0.0	8.2	0.0	0.0	34.3	0.0	9.8
	1935	0	0	18.6	0	0	36.9	0	2.4
	1936	0	0	18.8	0	0	44.9	0	2.2
	Average	0	0	15.2	0	0	38.7	0	4.8
2	1934	4.0	12.8	2.2	10.2	40.5	41.2	0	3.6
	1935	0	7.7	0	17.8	43.0	30.8	0	0
	1936	0	13.5	0	12.5	58.2	38.4	0	0
	Average	1.3	11.3	7	13.5	47.2	36.8	0	1.2
3	1934	20.0	0	0	7.1	60.0	25.0	4.2	0
	1935	30.3	.6	0	15.7	45.1	40.8	0	.6
	1936	29.3	0	0	11.6	46.5	49.3	0	0
	Average	26.5	.2	0	11.5	50.5	38.4	1.4	.2
4	1934	0	0	8.8	0	18.8	45.2	13.8	24.2
	1935	.9	0	13.2	0	32.0	29.7	13.5	9.6
	1936	0	0	10.5	0	33.1	29.5	6.9	4.2
	Average	.3	0	10.8	0	28.0	34.8	11.4	12.7
5	1934	0	0	0	15.6	0	61.8	0	27.9
	1935	0	0	0	19.1	0	27.0	0	18.8
	1936	0	0	0	12.7	0	21.4	0	10.4
	Average	0	0	0	15.8	0	36.7	0	19.0
6	1934	0	0	30.4	25.5	0	52.6	0	45.8
	1935	0	0	17.4	20.2	0	34.3	0	29.5
	1936	0	0	32.1	19.3	0	50.2	0	31.1
	Average	0	0	26.6	21.7	0	45.7	0	35.5
7	1935	.8	0	0	0	24.3	53.5	1.5	3.6
	1936	.3	0	0	0	29.5	26.9	0	.3
	Average	.6	0	0	0	26.9	40.2	.8	2.0
8	1935	0	0	0	0	.5	39.7	0	0
	1936	0	0	0	0	0	28.3	0	0
	Average	0	0	0	0	.3	34.0	0	0

TABLE 2.—*Reactions of 5 varieties of spring barley which differentiate 8 physiologic races of Ustilago hordei*

Race No.[1]	Reaction[2]—				
	Excelsior (C. I. 1248)	Hannchen (C. I. 531)	Lion (C. I. 923)	Nepal (C. I. 595)	Pannier (C. I. 1330)
8	R	R	R	R	R
1	R	I	R	R	R
7	R	R	R	I	R
4	R	I	R	I	I
5	R	R	I	R	R
6	R	I	I	R	R
2	R	R	I	S	R
3	I	R	I	S	R

[1] The race numbers are presented in the order given to facilitate comparison with the key to 8 physiologic races of *Ustilago hordei* that follows.

[2] R (resistant)=0–5 percent of smutted heads; I (intermediate)=6–35 percent; S (susceptible)=36 percent or more.

Key to eight physiologic races of Ustilago hordei

	Physiologic race
Lion resistant:	
Nepal resistant:	
Hannchen resistant	8
Hannchen intermediate	1
Nepal intermediate:	
Pannier resistant	7
Pannier intermediate	4
Lion intermediate:	
Nepal resistant:	
Hannchen resistant	5
Hannchen intermediate	6
Nepal susceptible:	
Excelsior resistant	2
Excelsior intermediate	3

TABLE 3.—*Number and distribution of physiologic races of barley covered smut in 200 collections from 26 States*

Location	Collections of race No. —								Total collections
	1	2	3	4	5	6	7	8	
	Number	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*
Arizona					1				1
California	1		1		34	1			37
Colorado						1			1
Georgia	3								3
Idaho		1	1		2	13			17
Illinois						1	2		3
Iowa			1			9			10
Kansas			1		1	1			3
Louisiana						1			1
Michigan						1			1
Minnesota	1					19			20
Missouri			1			1			2
Montana				1		2			3
Nebraska						9			9
New York						10			10
North Carolina	2					1			3
North Dakota				1		18			19
Oklahoma						1			1
Oregon		1			1				2
South Dakota						2			2
Texas	1								1
Utah					1	14			15
Virginia	7					1		1	9
Washington				1	17	5			23
West Virginia								1	1
Wisconsin						3			3
Total	15	2	5	3	57	114	2	2	200

The data in table 1 show, in general, a high degree of consistency in the percentages of infection with each of the races during the 2 or 3 years of the test. In some instances certain races produced a low percentage of smutted heads in certain varieties in 1934 but did not cause smut in these varieties in the following years. This may have been due to mixtures in the original collections that were screened out as a result of passage through selected hosts in the following years.

Despite the fact that environmental conditions were marked by unusual variations in temperature and precipitation in the three seasons in which the tests were conducted at Ithaca, the spore-suspension method of seed inoculation proved effective and the degree of smut infection was reasonably uniform.

Two facts are apparent from table 3: (1) The wide distribution of race 6 and (2) the predominance of race 5 in California and Washington.

WINTER VARIETIES AS DIFFERENTIAL HOSTS

In the fall of 1935, the 28 winter varieties or selections listed in table 4 were inoculated with the eight races of covered smut and sown in triplicated, systematically distributed rows at Statesville, N. C. In other respects the general conduct of the test was similar to that with the spring barleys. The results of the test are given in table 4. In general, these winter barleys displayed little clear-cut differential reaction to the smut races except that about two-thirds of them were resistant to race 2. The Nakano Wase selections, including Esaw, however, were moderately susceptible to race 2 and highly resistant or immune to all other races. The Smooth Awn selections 86 and 203 were the most uniformly resistant, showing less than 5 percent of heads smutted by any of the races. Unfortunately, Esaw and the Nakano Wase and Smooth Awn selections are highly susceptible to the brown loose smut (*Ustilago nuda* (Jens.) Kell. and Sw.) which is prevalent in the humid winter barley region.

Doubtless as a preface to further studies on the use of winter barleys as differential hosts, a better knowledge of the influence of winter injury on the incidence of covered smut should be acquired. Tisdale (*18*) and Faris (*3*) have shown that plants of certain winter barleys are more susceptible to winter injury when infected with covered smut than when not infected. In the experiment just described there were wide differences in the degree of winter injury sustained by the different varieties and this may have obliterated differential responses to the races of smut that otherwise would have been apparent.

TABLE 4.—*Percentages of smutted heads in 28 varieties of winter barley inoculated with 8 physiologic races of Ustilago hordei and grown at Statesville, N. C., 1935–36*

Race No.	Smutted heads in—													
	Alaska (C. I. 4106)	Beardless No. 6 (C. I. 2746)	Cusado (C. I. 895)	Esaw (C. I. 4690)	Gaddis (C. I. 6063)	Garren	Han River (C. I. 2163)	Kashmir	Kentucky No. 2 (C. I. 6148)	Kentucky Smooth Awn (C. I. 6021)	Khayyam (C. I. 1117)	Nakano Wase selection 33	Nakano Wase selection 68	Orel (C. I. 351)
	Pct.	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*
1	10.2	3.3	11.0	0.0	5.3	6.0	22.3	15.1	4.9	36.1	7.9	1.2	0.0	6.8
2	6.3	6.3	1.7	12.3	7.4	1.3	3.1	3.4	.2	9.2	2.4	7.3	10.9	2.1
3	14.4	16.2	6.0	.0	12.2	10.5	16.9	12.3	2.9	34.8	8.1	.0	.0	4.6
4	25.8	17.1	11.5	.0	15.8	13.3	21.2	18.6	7.7	24.6	11.4	.0	.0	16.8
5	40.8	2.6	19.4	.4	7.3	9.4	45.8	30.9	13.8	34.3	25.4	3.0	.6	24.7
6	40.9	5.8	22.3	2.2	5.1	6.5	47.3	28.2	12.8	28.8	26.8	.0	.0	20.3
7	30.5	14.0	28.1	.8	22.5	9.3	38.8	22.4	15.1	27.9	35.0	.0	1.2	22.6
8	39.5	4.9	26.1	1.9	9.2	6.8	32.2	33.1	12.7	28.6	26.6	.0	.0	19.3

TABLE 4.—*Percentages of smutted heads in 28 varieties of winter barley inoculated with 8 physiologic races of Ustilago hordei and grown at Statesville, N. C., 1935–36*—Continued

Race No.	Smutted heads in—													
	Pidor (C. I. 901)	Scottish Pearl (C. I. 277)	Smooth Awn selection 86	Smooth Awn selection 253	Smooth Awn selection 235	Squarehead (C. I. 252)	Tenkaw (C. I. 646)	Tennessee Winter (C. I. 3534)	Tennessee Winter (C. I. 3546)	Tennessee Winter (C. I. 6234)	Tennessee × Abyssinia (37) (C. I. 6236)	Texas Winter (C. I. 554)	Wisconsin Winter (C. I. 2159)	Woods Hooded (C. I. 6235)
	Pct.	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*
1	11.8	12.5	0.0	0.6	0.5	11.5	8.8	16.9	25.1	10.5	22.9	13.6	13.3	13.2
2	11.3	4.0	1.1	1.6	15.0	.9	2.2	2.9	6.8	.5	10.3	2.8	2.9	3.3
3	3.6	7.9	.0	.6	.0	6.8	11.8	12.8	23.7	6.0	16.7	10.1	12.1	11.1
4	19.1	19.4	1.4	.0	.0	21.7	16.6	20.0	37.4	11.9	20.8	16.0	16.6	19.2
5	27.6	25.5	4.7	3.1	5.1	24.7	28.1	25.3	45.7	7.9	29.6	26.1	39.3	10.6
6	39.6	35.0	1.7	.0	.0	36.2	35.8	31.6	57.2	8.5	37.4	38.1	33.3	14.9
7	13.6	31.1	.0	.0	4.8	32.8	37.3	33.2	45.8	10.2	29.4	32.1	34.2	21.1
8	16.5	37.7	.0	.0	2.5	30.7	29.3	27.6	54.5	7.7	35.5	29.1	30.1	11.7

Another factor that seems due to assume greater importance in future studies of physiologic races is the influence of environmental conditions on the response of the host after infection. The results of Faris (*3*) seem to indicate that after the smut has penetrated the seedling environmental conditions may influence the host response in a way other than through winter injury; and, as Faris notes, "the reaction of barley varieties to such environmental changes may not be the same." Aamodt (*1*), Smith (*13*), Rodenhiser and Holton (*12*), and Holton and Heald (*8*) have reported that environmental conditions after infection may affect the incidence of bunt in wheat.

DISCUSSION

Eight physiologic races of *Ustilago hordei* have been isolated on the basis of differences in their pathogenicity on eight varieties of barley. Although sufficient data are not yet available to permit a detailed discussion of the distribution of these races, it seems evident that the race designated No. 6 is the most widely distributed. It was found in 21 of the 26 States from which collections have been obtained. It was also the most frequently collected, occurring in 114 of a total of 200 collections. However, in California and Washington race 5 was outstanding, occurring 34 times in 37 collections from California and 17 times in 23 collections from Washington. The predominance of race 5 in California may be linked with the antiquity and survival of Coast barley in that State. As noted by Harlan and Martini (*7*), when North America was discovered there were no barleys here. The early Spanish missionaries introduced Coast barley into California about 1770, and it is still widely grown there. Of the 37 covered smut collections obtained from California, 26 came from Coast and the two Coast-type varieties Atlas and California Tennessee Winter. Twenty-three of these twenty-six collections proved to be race 5. The early importations of Coast seed in California may have harbored this particular race of smut, which has survived and spread with its susceptible and popular host.

Despite the apparent restriction of certain smut races to limited areas, the probability that the different races will be spread through wind dissemination of spores and through the interchange of infected seed makes it highly desirable to breed barleys resistant or immune to all the known races of smut. To date Pannier (C. I. 1330) has proved highly resistant or immune to seven races and only moderately susceptible to one. *Hordeum deficiens* (C. I. 668–1) and *H. intermedium* (C. I. 4377), used only in the test of 1934, were highly resistant or immune to the six races occurring in the collections of that year. These two and Pannier also have proved highly resistant or immune to two races of the black loose smut (*Ustilago nigra Tapke*) of barley recently described (*14, 16*). In these experiments no smut has been observed in Hillsa (C. I. 1604) and Lyallpur (C. I. 3403), yet the former was inoculated with 65 collections of *U. hordei* in 1933 by Faris and both were inoculated with eight races of *U. hordei* and two of *U. nigra* in 1935 by the writer (*16*). In the light of the results of the present investigation, it appears that physiologic races of *U. hordei* are not more numerous than those of other small-grain smuts and that the breeding of barleys for resistance to covered smut should not be hampered by lack of resistant parental material.

SUMMARY

Eight physiologic races of *Ustilago hordei* were found in 200 collections from 26 States. Race separation was based on differences in pathogenicity on five varieties of spring barley.

The most widely distributed race was collected in 21 of the 26 States. It was also the most generally prevalent race, occurring 114 times in the 200 collections.

In California and Washington another race was conspicuously prevalent and widespread. It occurred 51 times in 60 collections from these States.

Under the conditions of a 1-year test with 28 winter varieties or selections, little clear-cut differential host response to the 8 races of covered smut was obtained. Marked differences in varietal response to winter injury occurred. A better knowledge of the influence of this factor on the incidence of covered smut in winter barleys is needed.

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IMPROVEMENTS IN DETERMINATION OF OIL DEPOSIT ON SPRAYED FOLIAGE [1]

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INTRODUCTION

A number of methods for the determination of oil deposit on sprayed foliage have been proposed during the last 7 years, but nearly all the reliable ones have been applicable only in the determination of the highly refined petroleum oils. Success with such oils has depended upon the fact that they remain comparatively inert, whereas a large proportion of the natural substances extracted from leaves is destroyed by treatment with strong mineral acid (*4*, *5*, *7*).[2] Under such conditions it is possible to collect and measure the oil in Babcock bottles, and with a knowledge of the surface area of the extracted foliage, to calculate the deposit in terms of the quantity of oil retained per unit of leaf area.

In the case of the less refined petroleum oils (*3*), however, the authors found it necessary to carry check samples, to which known quantities of particular oils had been added, along with the unknown samples, for the purpose of correcting the results both for the plant substances undestroyed by the acid and for certain unsaturates eliminated from the petroleum oil itself by the acid treatment. These operations were no more time consuming than the average chemical determination. In a subsequent attempt to develop an even simpler method, applicable also in the analysis of fatty oils, Dawsey, Cressman, and Hiley (*4*) tried to standardize emulsions upon the basis of the quantities of oil retained by wax-coated plates, but found that the deposit on plate surfaces was not always proportional to that retained by a leaf surface. It was concluded that accurate results were best achieved by oil extraction directly from the foliage, and since then work has been carried forward on such a basis.

The purpose of the investigations described in this paper was to develop improved methods for the determination of oil deposit on foliage which would be applicable to oils of animal and vegetable origin as well as to the nonvolatile petroleum oils. With this objective experiments were carried out to ascertain (1) which was the best solvent to employ in recovering oil from sprayed foliage, (2) the optimum conditions for recovery of 100 percent of the oil, or the total deposit, and (3) the accuracy obtainable under the optimum conditions for oil recovery.

EXPERIMENTAL PROCEDURE

All the tests were performed with leaves from plants of the Old Rose variety of chrysanthemum (*Chrysanthemum hortorum*) which

[1] Received for publication June 22, 1937; issued December 1937.
[2] Reference is made by number (italic) to Literature Cited, p. 701.

had been grown in the greenhouse in pots and allowed to reach a height of about 12 inches. Applications to sprayed foliage were made with emulsions stabilized with bone glue in the proportion of 6 g of glue to 100 cc of oil. The precision sprayer and the spraying methods employed have been described previously (4).

EXTRACTION EFFICIENCY OF DIFFERENT SOLVENTS ON NATURAL LEAF SUBSTANCES

The first tests were carried out to determine which of the more common solvents was best adapted for extracting oil from chrysanthemum foliage. It was apparent that the most suitable solvent would be the one in which the spray oil was readily soluble and yet which would dissolve the least material from the leaves.

Table 1 gives a comparison of the quantities of materials extracted by different solvents in the treatment of both powdered leaves and fresh leaf disks in a conventional type of apparatus. To prepare the powdered samples, unsprayed leaves were dried in an electric oven at 110° C., powdered in a mortar, and 0.5000-g samples were weighed out and placed in the thimbles of an A.S.T.M. (American Society for Testing Materials) rubber-extraction apparatus. Extraction was allowed to proceed until the liquids in the siphon cups were colorless. The extracts were filtered into weighed flasks, the solvents evaporated off, and the residues dried to constant weight at 110°. For the leaf-disk samples, 200 fresh disks of 1 cm diameter were cut per sample from the unsprayed plants. It was found that this number of disks, when dried and powdered, weighed 1.068 g. The fresh disks were extracted in a Bailey-Walker extractor for 3 hours, the solvents evaporated in the original weighed extraction flasks, the last traces of volatile substances taken off in a vacuum desiccator at a pressure of 2 to 3 mm of mercury, and the residues determined at constant weight.

TABLE 1.—*Relative quantities of plant substances extracted from dry, powdered leaves and from fresh, green chrysanthemum-leaf disks by different solvents*

Solvent	Powdered leaves		Fresh leaf disks	
	Color of extract	Residue	Color of extract	Residue
		Mg		*Mg*
Petroleum ether	Yellow	44.9	Light yellow	17.1
Carbon disulphide	Dark green	76.9	Brown	34.7
Carbon tetrachloride	do	80.3	Yellow	48.6
Ethyl ether	do	83.5	Green	49.1
Benzene	do	83.9	Yellow	38.2
Methylene chloride	do	90.1	do	
s-Dichloroethylene	do	112.8	do	37.5

Although the powdered leaves were extracted in the A.S.T.M. apparatus and the fresh leaf disks in the Bailey-Walker apparatus, the two sets of results are at least indicative of the relative quantities of leaf substances extractable, since the residues are calculated upon the basis of 1.068 g of dry leaf material continuously extracted for approximately the same period of time.

It is evident that by drying and grinding the leaves before extraction considerably more nonvolatile material is extracted than when green disks are used. In both types of treatment minimum quantities

were taken out by petroleum ether; so this solvent appeared to be the most promising one. In the extraction of the disks the petroleum ether showed only a light-yellow color, indicating that very little chlorophyll had been extracted. This is in keeping with the fact that chlorophyll is contained within the leaf cells, and if the cell walls are not broken practically none is extracted by this solvent. Such small quantities of substances as were obtained with petroleum ether from the fresh disks must, therefore, have consisted of natural waxes and oils from the surface of the leaves. It was concluded from this experiment that petroleum ether would be the most suitable solvent to use in extracting the oil deposit from sprayed foliage.

Rohrbaugh (*7*) claimed petroleum ether to be a superior solvent in the extraction of petroleum oils from dry, powdered citrus foliage.

METHOD OF EXTRACTION AS AFFECTING OIL RECOVERY

English (*6*) stated that oil was completely recoverable from Satsuma orange foliage in a limited number of washings on leaf disks, because subsequent extraction in a Soxhlet apparatus failed to show additional quantities of oil. Later Dawsey (*3*, *5*) made improvements in the English method, based upon the successive washing of disks with ethyl ether, as used in the determination of oil deposit on the foliage of camphor-tree, Satsuma orange, pecan, and chrysanthemum. Rohrbaugh (*7*), on the contrary, claimed that simply washing unground citrus leaves in such a solvent was insufficient for complete recovery.

It was therefore considered desirable to study some of the different methods of extraction and thereby determine the optimum conditions for complete recovery of the deposit from sprayed foliage. To this end experiments were performed to determine the efficiency of oil recovery in washing leaf disks a limited number of times, and also in continuous extraction as is best carried out in a standard type of extraction apparatus.

WASHING METHOD

The first of these tests was a simple washing experiment with ethyl ether and carbon tetrachloride as solvents. Samples containing 200 disks of 1 cm diameter were taken from unsprayed plants and from plants sprayed with a 2-percent emulsion of a highly refined petroleum oil. They were extracted in 125-cc flasks by washing four times with 35 cc of solvent per washing, after which the residues in the total extracts were determined. All residues were determined on a volume basis in Babcock bottles except the initial extractions with carbon tetrachloride, which were determined by weighing. The specific gravity of the oil was 0.8512 as used in converting weight to volume. The oil from the samples of sprayed foliage was calculated by subtracting therefrom the mean of the residues extracted from the unsprayed foliage. Afterward the exhausted leaf disks were dried and reduced to powder, and the washing treatment was repeated to recover any remaining oil. Table 2 shows in the case of both solvents the quantities of residue initially extracted and those extracted in the second treatment.

TABLE 2.—*Extraction efficiency in washing the oil deposit from mixed leaf disks with ethyl ether and carbon tetrachloride*

ETHYL ETHER EXTRACTIONS

Sample no.	Residue from initial washings				Residue from final washings			
	Sprayed disks	Unsprayed disks	Total oil recovered		Sprayed powder	Unsprayed powder	Total oil recovered	
	*Mm*³	*Mm*³	*Mm*³	*Percent*	*Mm*³	*Mm*³	*Mm*³	*Percent*
1	58.7	4.3			9.2	4.2		
2	59.6	4.3			9.1	3.9		
3	60.8	4.6			8.3	3.7		
4	61.2	4.5			9.3	4.3		
Mean	60.1	4.4	55.7	91.8	9.0	4.0	5.0	8.2

CARBON TETRACHLORIDE EXTRACTIONS

	Mg	*Mg*	*Mg*					
1	47.9	7.0			9.6	2.4		
2	51.9	7.1			11.4	3.1		
3	51.1	7.0			10.7	3.3		
4	53.3	6.6			12.5	2.7		
Mean	51.1	6.9	44.2	86.4	11.1	2.9	8.2	13.6

The total deposit as obtained with ethyl ether agrees closely with that obtained with carbon tetrachloride, the two figures being 60.7 and 60.1 mm³, respectively, when calculated from the data in the table. In the four initial washings ethyl ether was slightly more efficient, but since neither solvent recovered the oil completely, it was concluded that more drastic extraction measures were necessary.

As in this example with chrysanthemum leaves, it is improbable that extractions carried out by Dawsey (*3*), in earlier work on camphor-tree and chrysanthemum foliage, recovered all the deposit. Washing disk samples four times with ethyl ether, however, is seen to give a good approximation of the total deposit on the foliage, and it is unlikely that the general conclusions regarding the insecticidal action of oil sprays drawn in previous work (*1*, *2*) are seriously in error.

CONTINUOUS EXTRACTION

A continuous method of extraction, with petroleum ether as the solvent, was tried next. Samples of green leaf disks taken from plants sprayed with a 2-percent oil emulsion, and from unsprayed plants, were prepared as before and immediately extracted with petroleum ether in the A. S. T. M. rubber-extraction apparatus. After continuous refluxing for 2 hours, the solvent was evaporated down on the water bath, the extracts were transferred to weighed flasks, and the residues were heated to constant weight at 110° C. The residues from the initial extracts were weighed. They were then transferred from the weighing flasks to Babcock bottles and the oil in each sample was redetermined according to the previously developed volumetric method (*3*). Thus, a direct check was obtained upon the accuracy of the weighing method. Both the Babcock measurements and the results obtained in weighing are included in table 3. After the initial extractions, the exhausted leaf disks were dried, reduced to powder, and reextracted to determine whether all traces of the spray oil had been taken out during the first operation. The residues from the

second extractions were estimated by the Babcock method, and these figures are also shown in table 3.

TABLE 3.—*Oil-recovery efficiency of petroleum ether in continuous extraction on fresh leaf disks in A. S. T. M. extraction apparatus*

Sample no.	Residue from initial extraction								Residue from final extraction, Babcock determination			
	Weight determination				Babcock determination							
	Sprayed disks	Unsprayed disks	Oil recovered		Sprayed disks	Unsprayed disks	Oil recovered		Sprayed powder	Unsprayed powder	Oil recovered	
	Mg	*Mg*	*Mg*	*Pct.*	*Mm³*	*Mm³*	*Mm³*	*Pct.*	*Mm³*	*Mm³*	*Mm³*	*Pct.*
1	29.7				32.4				2.5	1.6		
2	39.3	4.6			43.5	3.4			1.7	1.9		
3	38.2	4.5			43.4	3.5			1.8	2.2		
4	38.2	3.9			42.8	3.2			1.7	2.1		
Mean	36.4	4.3	32.1	100.0	40.5	3.4	37.1	98.4	1.9	2.0	0	0

This experiment showed that it was possible to recover 100 percent of the oil deposit, provided fresh leaf disks were continuously extracted in a standard type of apparatus for 2 hours with petroleum ether. This was proved by the fact that further drying, grinding, and extracting of the previously exhausted disks did not give any more oil than was obtained from the unsprayed blanks that were run simultaneously. There was considerable variation from sample to sample, but the quantities of oil as checked by the Babcock method indicated that the weight determinations were reliable. The results of the Babcock determination were slightly lower than those obtained by the weighing method, but this was to be expected since small quantities of oil may have been lost in transferring the residues from the weighing flasks to the Babcock bottles.

TABLE 4.—*Effect of lapse of time between spray application and extraction upon the efficiency of oil recovery from plant foliage*

24 HOURS AFTER SPRAY APPLICATION

Sample no.	Residue from initial extraction								Residue from final extraction, Babcock determination			
	Weight determination				Babcock determination							
	Sprayed disks	Unsprayed disks	Oil recovered		Sprayed disks	Unsprayed disks	Oil recovered		Sprayed powder	Unsprayed powder	Oil recovered	
	Mg	*Mg*	*Mg*	*Pct.*	*Mm³*	*Mm³*	*Mm³*	*Pct.*	*Mm³*	*Mm³*	*Mm³*	*Pct.*
1	62.4	7.6	54.4		66.8	4.2	63.0		1.6	1.7		
2	61.8	8.1	53.8		66.7	3.5	62.9		1.1	1.0		
3	64.2	8.0	56.2		69.8	3.6	66.0			1.4		
4	59.4	8.2	51.4		64.2	4.0	60.4		1.4	.9		
Mean	62.0	8.0	54.0	100.0	66.9	3.8	63.1	99.5	1.4	1.3	0.1	0

6 DAYS AFTER SPRAY APPLICATION

1	55.2	8.3	47.0		56.9	3.7	53.3		1.3	1.3		
2	53.1	8.2	44.9		56.7	4.1	53.1		1.3	1.3		
3	49.9	8.0	41.7		54.0	3.4	50.4		1.2	1.3		
4	56.0	8.3	47.8		59.2	3.2	55.6		1.5	1.1		
Mean	53.6	8.2	45.4	84.1	56.7	3.6	53.1	83.8	1.3	1.3	0	0

Further experiments were conducted to ascertain whether the total deposit could be recovered by continuous extraction with petroleum ether when the extraction was delayed and larger deposits were used (table 4). In these tests a set of plants was divided, some of the plants being sprayed with a 5.0-percent petroleum oil emulsion while others were left unsprayed. The foliage from half the sprayed plants was analyzed about 24 hours after application and the other half were set aside in the greenhouse for analysis 6 days later. In both cases extractions were performed on samples containing 100 disks clipped not more than 3 to 4 hours before extraction. Although only 84.1 percent of the original oil deposit present could be found on the plants 6 days after spraying, reextraction of the exhausted samples, after drying and grinding, showed the oil recovery to be 100 percent. It is thought that the reduction in deposit between the first and the sixth day must have been due to volatilization together with weathering (*8*) of the oil on the plant.

EFFECT OF TREATMENT OF LEAVES BEFORE EXTRACTION

In further experiments on processing leaves before extraction, it was found that only 83 to 88 percent of the total deposit could be recovered during a 2-hour continuous extraction with petroleum ether in the A.S.T.M. apparatus when the disks were dried but not ground to powder. It appears, therefore, that mere drying of the leaves makes the oil even more difficult to extract, so that reduction of the material to a powder then becomes imperative, whereas if the leaves are extracted when fresh, continuous washing is sufficient for complete extraction of the oil deposit. Elimination of the necessity for drying and grinding thus simplifies the work involved in routine analysis.

CHECK ANALYSES

One experiment was carried out with refined corn oil, a typical vegetable oil, to ascertain approximately the errors likely to occur in handling samples during analysis.

Eight samples, each containing 200 disks from unsprayed foliage, were prepared. To four of the samples known weights of corn oil were added; the remaining four samples were used as blanks. The extractions were made under the previously determined optimum conditions, followed by determination of the weights of residues and calculation of the quantities of oil known to be present. Table 5 shows the differences between the calculated quantities of oil present and those known to have been added. The differences calculated on the basis of the amounts of oil present are not more than ± 2 percent. The largest errors occurred in the first two samples where the quantities of oil present were small, but if differences between calculated and known quantities of oil are considered in terms of weight, then it is seen that they fall within the variations occurring among blank samples; hence, the main source of error must be ascribed to variations in the quantities of natural leaf substances in the oil-containing samples rather than to losses of oil in handling.

TABLE 5.—*Calculated quantities of corn oil recovered from samples by petroleum ether extraction as compared with known quantities of the oil added to the samples*

Sample no.	Oil added	Total residue extracted	Leaf substances present [1]	Calculated oil present	Error
	Mg	*Mg*	*Mg*	*Mg*	*Percent*
1	24.9	36.4	11.5	25.3	+1.6
2	56.5	66.8	10.3	55.7	−1.4
3	113.4	124.9	11.5	113.8	+.4
4	182.3	193.5	11.2	182.4	.0
Mean			11.1		

[1] In the blank samples the quantities of leaf substances were 11.0, 10.6, 12.0, and 11.4 mg, with a mean of 11.3 mg.

It is at once apparent that the accuracy in the petroleum ether extraction method for determination of oil deposit depends primarily upon having the same quantities of leaf substances in both blank and oil-containing samples, so that when the blank weights are subtracted from the weights of the oil samples a true figure is obtained for the oil content. This is best accomplished by running blanks simultaneously with oil samples, since blanks are known to vary, depending upon the length of time they are extracted. Moreover, the error in calculating the oil deposit may be larger for small deposits than for large deposits. For example, the following residues were extracted with petroleum ether in a test on unsprayed chrysanthemum foliage where the samples consisted of 200 disks, the time of extraction was 2 hours, and the Bailey-Walker apparatus was used:

Residues, mg	18.4	18.9	20.6	17.6	16.8	18.9	17.5	M=18.4
Difference from mean, mg	0	+.5	+2.2	−.8	−1.6	+.5	−.9	

The extreme difference from the mean was +2.2 in the third sample. If this sample had 30 mg of oil in it, the error introduced in analysis would have been about 7 percent; but if this sample contained as much as 300 mg of oil, the error would have amounted to only 0.7 percent. In practice the petroleum ether method has been found to show extreme errors within this range when a number of samples of sprayed, mixed disks are analyzed together with blank samples. Ordinarily with an average-sized deposit of foliage, however, the error in analysis is small, and may be neglected in view of the fact that very large differences in oil deposit, due to the coverage factor in spraying, completely obscure small errors such as are detectable in the foregoing type of check analysis.

DISCUSSION

Although most of the experimental data were obtained with a refined petroleum oil of 94-percent unsulphonatable residue, the petroleum ether extraction method for determination of oil deposit on foliage, when carried out under appropriate conditions, is applicable to nearly all kinds of nonvolatile insecticidal oils, including the vegetable, animal, and less refined petroleum oils, the last named of which are appreciably attacked by acid treatment in the hitherto employed Babcock methods of analysis. A limitation exists, however, in the determination of semivolatile oils such as pine oil, orange oil,

or kerosene, inasmuch as evaporation losses take place during analysis. The analytical procedure can be followed with standard pieces of laboratory equipment, since it embraces only four operations: (1) Cutting leaf disks in preparation of samples, (2) extracting samples with petroleum ether, (3) evaporating the solvent, and (4) drying residues to constant weight. The extraction can be made practically automatic with the aid of an electrical time switch, so that the labor is expended chiefly in cutting the leaf disks and weighing the residues.

Although the method has been developed primarily for determination of oil deposit on chrysanthemum, it is possible that the same principles can be applied to other kinds of foliage. For example, apple leaves are known to give very much smaller blank residues than chrysanthemum leaves, on the basis of equivalent foliage areas; so the method may be applicable in the analysis of apple foliage with even greater accuracy. Whether the method can be used on citrus is not known, but it is thought that waxy leaves will give larger blanks, thereby lessening the accuracy somewhat.

SUMMARY

An improved weighing method is described for determination of oil deposit on chrysanthemum foliage after spraying with emulsions. The method is applicable to nearly all kinds of nonvolatile insecticidal oils, including both petroleum oils and fatty oils. The procedure consists of four steps: (1) Cutting leaf disks in preparation of samples, (2) extracting samples, (3) evaporating the solvent, and (4) drying residues to constant weight. Recovery of oil from chrysanthemum foliage is 100 percent.

Petroleum ether was found to be the most suitable solvent to use in extraction, because it dissolved smaller quantities of the natural plant substances present on the foliage than other solvents tested.

All the oil could not be extracted by simply washing disks a limited number of times, but when samples contained freshly cut disks and extraction was carried out in a standard type of apparatus for 2 hours, recovery was complete. Drying and grinding of the foliage before extraction was not necessary for complete recovery of deposit even 6 days after spray application.

The accuracy attainable by the method is influenced by the variation in quantities of natural leaf substances extracted from the same number of leaf disks, from sample to sample, and depends upon the size of the oil deposit being measured as compared with the quantity of leaf substances present. The error in calculating low deposits is larger than the error in calculating high deposits. With average-sized and higher oil deposits the error in analysis is small and may be neglected, since very large differences, due to the coverage factor in spraying, obscure any errors detectable by the method of analysis when used in actual practice.

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INTERRELATIONSHIPS OF EGG PRODUCTION FACTORS AS DETERMINED FOR WHITE LEGHORN PULLETS [1]

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INTRODUCTION

It has long been recognized that the egg-producing ability of the domestic fowl depends on a number of inherited and noninherited factors. Since the early attempts to identify and describe these factors a voluminous literature has accumulated. Jull (*7*) [2] has compiled a bibliography which includes the larger proportion of papers published in this field. It must be pointed out, however, that only a few such papers deal with the actual establishment of criteria for these factors. Most of the papers present statistical analyses of observables, described by arbitrarily selected criteria.

Of the attempts to justify the use of one or another measurement on other than empirical grounds, a notable contribution is that of Knox, Jull, and Quinn (*8*), who studied the interrelationships between a number of different heritable and nonheritable factors as well as their relation to the annual egg record. Their conclusions were somewhat at variance with some of the work reported earlier, such as that of Hays and Sanborn (*6*), and with some of the work published since (Lerner and Taylor (*9, 10, 11*)). The latter have suggested the use of a number of criteria for the measurement of certain of the factors affecting egg production. The present paper deals with some extensions and further ramifications of these.

Essentially, there are two types of genetic factors that enter into the expression of the egg-laying ability of the fowl. The first type affects the period of time during which the bird is in lay, and includes maturity, persistency, and pauses due to broodiness or other causes. The second type determines the intensity or rate of production of the birds when in lay.

Maturity has been found to be adequately measured by the age at first egg (Warren (*14*); Knox, Jull, and Quinn (*8*); Hays (*5*)). Persistency has been defined differently by different workers, and the grounds on which age or date at last egg are suggested as criteria for its measurement have been discussed by Lerner and Taylor (*10*). Pauses as yet have been defined only arbitrarily, various measurements of pause having been used by Hays (*3, 4*) and others. The measurement of rate has also been a controversial issue, particularly in the relation of rate to pauses. The use of net rate of production (number of eggs divided by number of days in the laying period considered, less the number of days broody and in pause) has been discussed by Lerner and Taylor (*9*). The application of these criteria and the effect of the observables they measure on egg production form the subject for the analysis presented in this paper.

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[2] Reference is made by number (italic) to Literature Cited, p. 712.

MATERIALS AND METHODS

The trap-nest records of the Single-Comb White Leghorn flock of the Poultry Division were used for the raw data of this analysis. The first-year laying records of two generations of birds were examined and only those of individuals that answered the following requirements were selected: (1) Birds that had completed their full first biological laying year. This eliminated all birds that died or were otherwise disposed of before undergoing a complete molt at the end of the first laying year. (2) Birds that did not pause; that is, had no periods of 7 consecutive days of nonproduction during this time. This requirement was set up to eliminate the question of pause from consideration as far as possible until such time as more precise criteria of pause than those now used become available. It is recognized that the establishment of such a requirement has a certain element of arbitrariness in it, but it is likely that only a few, if any, birds which are genetically pausers would be included in the selected population when 7 days is used as the standard for pause. A possible shortcoming of this method lies in the exclusion of some nonpausing birds and the consequent reduction in the number of birds represented in the populations studied.

These requirements provided a highly selected population of fairly superior layers. Since the hatching season for the 2 years studied was confined to March and April, and the management was uniform throughout, environmental variation may be considered as having been greatly restricted. The analyses for the 67 birds of the 1933 series were made separately from the analyses for the 100 birds of the 1934 series, so that duplicate figures for the 2 years are presented.

The statistical methods used are those described by Ezekiel (*2*), except for the calculation of the coefficient of multiple correlation, which was made in accordance with the method of Wallace and Snedecor (*13*), and the analysis of variance, which was performed in accordance with Snedecor's manual (*12*). A fuller discussion of the various measures of part and partial correlation used will be made in conjunction with the data presented.

FACTORS CONSIDERED

Two measures of annual egg production were used: (1) The production during the 365 days immediately following the first egg, which is the measure most commonly used in breeding and in experimental practice; and (2) the production during the whole of the first biological laying year, from the first egg at sexual maturity to the last egg laid at the onset of fall molt. The latter measure reflects more accurately the inherent ability of the bird to lay, since it lacks the arbitrary element introduced in the first measure.

Since the population selected was nonpausing and nonbroody, the only time factors involved are maturity and persistency. Age at first egg was adopted as the measure for the former and age at last egg for the latter. Material presented elsewhere (*11*) indicates that for the population studied the two measures are equivalent so far as the proportion of variance that is genetic in nature is concerned. Although on the basis of susceptibility to environmental differences, date of last egg was found to be a superior measure of persistency, age at last egg

was used here by analogy with the measurement used for sexual maturity.

The type of measurement for rate of production presented the most difficulty. Though on the basis of considerations previously outlined (*9*) net rate was to be used, the essential point of importance was the selection of the period of time to be used for the determination of rate. As a first step, the calendar year was divided into three 4-month periods, designated winter (November–February), spring (March–June), and summer and fall (July–October). For the sake of brevity the last period will henceforth be referred to as summer.

The rate of production for each bird for each of these periods was calculated and then used individually in combination with the two measures of the time factor. Rate was also measured by single monthly periods but these calculations are not presented, since the establishment of broader principles rather than details is desired here.

As the first step in the analysis proposed, the heritability of the factors to be studied was determined. The simplest way of approaching this problem is through the determination of the relative variance in the observables studied which can be assigned to the differences between the sires of the pullets used in the study. While such variance would represent only a portion of the total genetic variance, a relative estimate of the sires' contribution which finds its expression in the phenotype of the daughters is made. Since the number of different sires involved in the 1933 series was somewhat out of proportion to the number of daughters, the analysis of variance was carried out only for the 1934 series. The results of this analysis are presented in table 1. The lower limit of significance for the degrees of freedom involved is an F value of between 1.65 and 1.88, and it may readily be seen that with two exceptions all the values are highly significant. Winter rate is found to have border-line significance, but undoubtedly passes the test of heritability since only a portion of the genetic variance is

TABLE 1.—*Analysis of variance of the dependent and independent variables with respect to sires, 1934 series* [1]

Source of variance	Degrees of freedom	365-day production		Biological laying-year production		Age at first egg	
		Total squares	Mean square	Total squares	Mean square	Total squares	Mean square
Total	99	101,093		183,903		86,275	
Between means of sires	19	45,786	2,409.8	84,834	4,464.9	22,584	1,188.6
Within means of sires	80	55,307	691.3	99,069	1,238.4	63,691	796.1
F			3.49		3.61		1.49

Source of variance	Age at last egg		Winter rate		Spring rate		Summer rate	
	Total squares	Mean square	Total squares	Mean square	Total squares	Mean square	Total squares	Mean square
Total	183,155		5,511		5,211		6,178	
Between means of sires	76,039	4,002.1	1,698	89.4	2,348	123.6	2,367	124.6
Within means of sires	107,116	1,338.9	3,813	47.7	2,863	35.8	3,811	47.6
F		2.99		1.88		3.45		2.62

[1] F at 5-percent point —1.88; F at 1-percent point —2.42.

represented here. The lack of significance in age at first egg is, however, surprising, not only because there is no question as to its genetic nature (*5, 14*), but also because at least one of the pairs of genes concerned in its determination is known to be sex-linked. Thus the greater portion of the genetic variance exhibited in the daughters should be ascribable to the influence of the sire. A more complete test made on a larger population where pausing pullets were also included in the analysis indicated that a genetic basis for age at first egg cannot be questioned (*11*).

CORRELATION ANALYSIS

The coefficients of simple correlation between the various observables studied are presented in table 2. Instead of the standard errors of these coefficients, the values of $\bar{r}$, representing values most probably true for the universe from which the sample was drawn and calculated in accordance with the formula presented by Ezekiel (*2*), are given. These values serve as an adjustment for the size of the sample used, at the same time indicating the true magnitude of various correlation coefficients with a greater degree of accuracy.

TABLE 2.—*Zero order coefficients of correlation, 1933 and 1934 series*

Variables correlated	1933 series		1934 series	
	r	$\bar{r}$	r	$\bar{r}$
365-day production, age at first egg	−0.348	−0.329	−0.387	−0.376
365-day production, age at last egg	.431	.417	.542	.536
365-day production, winter rate	.495	.484	.543	.537
365-day production, spring rate	.705	.699	.561	.555
365-day production, summer rate	.565	.556	.463	.454
Biological laying-year production, age at first egg	−.430	−.416	−.381	−.369
Biological laying-year production, age at last egg	.592	.583	.691	.687
Biological laying-year production, winter rate	.466	.453	.502	.495
Biological laying-year production, spring rate	.594	.586	.359	.346
Biological laying-year production, summer rate	.484	.472	.380	.367
Age at first egg, age at last egg	.131	.045	.151	.114
Age at first egg, winter rate	.087	0	.138	.095
Age at first egg, spring rate	.020	0	.133	.089
Age at first egg, summer rate	.142	.071	.029	0
Age at last egg, winter rate	.137	.063	.177	.145
Age at last egg, spring rate	.159	.100	.271	.252
Age at last egg, summer rate	.243	.212	.090	0
Winter rate, spring rate	.589	.581	.471	.463
Winter rate, summer rate	.348	.329	.212	.187
Spring rate, summer rate	.745	.740	.905	.904

It is evident from table 1 that the five independent factors exercise an influence on the annual record, measured either by the 365-day or by the biological laying-year production. The time factors are relatively independent of each other, the coefficients of correlation between age at first egg and age at last egg being 0.045 and 0.114 for the two respective series. Age at first egg also shows very low correlation with the three measures of rate, the coefficients varying in magnitude from zero to 0.095. Age at last egg, on the other hand, shows somewhat higher coefficients of correlation with the rate measurements. However, the two series do not give exactly the same results. In order of magnitude, age at last egg is most closely correlated with summer rate

and least with winter rate for the 1933 series, while for the 1934 series it shows a zero coefficient with summer rate and the highest value of 0.252 with spring rate.

Correlation coefficients between rates during the different seasons also show some variation. The highest correlation obtained is between spring and summer rate, followed by that between winter and spring rate, the lowest coefficient being that between winter and summer rate. The latter is, of course, to be expected, since the two periods do not follow one another on the calendar scale, as is the case with the other correlations.

Table 3 presents coefficients of multiple correlation with two and three independent factors at a time and the two production measures in turn as the dependent variable. The most probable values of the coefficients for the universe are once more presented instead of the standard errors, and these values are used for calculations leading to the results presented in the subsequent tables.

TABLE 3.—*Coefficients of multiple correlation, 1933 and 1934 series*

Independent variables	1933 series				1934 series			
	365-day production		Biological laying-year production		365-day production		Biological laying-year production	
	R	$\overline{R}$	R	$\overline{R}$	R	$\overline{R}$	R	$\overline{R}$
Age at first egg, age at last egg	0.594	0.577	0.782	0.775	0.721	0.714	0.848	0.844
Age at first egg, winter rate	.632	.616	.663	.650	.716	.709	.678	.670
Age at first egg, spring rate	.793	.785	.740	.731	.730	.723	.562	.550
Age at first egg, summer rate	.711	.701	.699	.688	.612	.602	.545	.531
Age at last egg, winter rate	.616	.600	.708	.697	.708	.701	.791	.786
Age at last egg, spring rate	.775	.767	.779	.771	.693	.685	.713	.706
Age at last egg, summer rate	.641	.627	.688	.676	.683	.675	.760	.754
Age at first egg, age at last egg, winter rate	.757	.744	.891	.885	.883	.879	.958	.956
Age at first egg, age at last egg, spring rate	.876	.869	.932	.929	.864	.860	.878	.874
Age at first egg, age at last egg, summer rate	.791	.780	.885	.879	.836	.830	.909	.906

It may be noted that the addition of any factor one at a time raises the values of the coefficients of correlation above those obtained with two variables. Using three independent factors at a time further increases the magnitude of the coefficients of correlation obtained. The squares of these coefficients are used as the coefficients of total determination (table 4). They indicate the percentage of variance in the annual record which is accounted for by the variance in the three independent factors.

No uniformity can be observed as to the magnitude of the total determination with respect to the measures used for rate. Thus in the 1933 series the highest coefficients obtained are those for which spring rate is used, accounting for 75.6 percent of the variance of the 365-day record and 86.3 percent of that of the biological laying-year production. In the 1934 series, however, it is the winter rate which gives the highest determination, accounting for 77.3 and 91.4 percent of the variance for the two respective measures of an annual production. On the other hand, the lowest coefficients are obtained when winter rate is used with respect to the 365-day production and summer

rate with respect to biological laying-year production in the 1933 series. In the 1934 series summer rate in the first case and spring in the second yielded the lowest values.

TABLE 4.—*Coefficients of total determination, 1933 and 1934 series*

Dependent variable	Independent variables	1933 series		1934 series	
		Coefficient of total determination	Standard error of estimate	Coefficient of total determination	Standard error of estimate
365-day production	Age at first egg, age at last egg, winter rate.	0.553	19.8	0.773	15.2
	Age at first egg, age at last egg, spring rate.	.756	14.6	.739	16.3
	Age at first egg, age at last egg, summer rate.	.609	18.4	.689	17.8
Biological laying-year production	Age at first egg, age at last egg, winter rate.	.783	16.9	.914	12.9
	Age at first egg, age at last egg, spring rate.	.863	13.5	.764	21.4
	Age at first egg, age at last egg, summer rate.	.773	17.3	.821	18.6

The coefficients of total determination obtained by Knox, Jull, and Quinn (*8*) fall within the range of values presented here. These workers, however, used six independent factors, and the highest coefficient they obtained with a nonselected population of White Leghorns was 0.766. Thus for the above coefficient, date of hatch, date of first egg, age at first egg, length of winter pause, and number of eggs laid in August and September were used. Date of hatch is not a heritable factor; age at first egg and date of first egg are duplicating measures of maturity since the coefficient of simple correlation reported by Knox, Jull, and Quinn between these two variables is 0.901. The number of eggs laid to March 1, though designed to measure intensity, is also a measure of pause, showing a correlation coefficient with length of winter pause of -0.824. Similarly, production in August and September falls short of being an adequate measure of persistency, since it is probable that the element of rate influences it, although no correlation coefficients to test this point are presented by Knox, Jull, and Quinn.

When these workers used only three independent measurements, purporting to measure the same observables as used in this paper, a coefficient of multiple determination of 0.745 was obtained for a White Leghorn flock and of 0.785 for a Rhode Island Red flock, values which are of the same general magnitude as the ones reported here. It should be noted that these independent observables measuring rate (production to Mar. 1) and persistency (August and September production) both form a part of the annual record, thus tending to make the coefficients reported spurious to a degree. In the case of rate as measured here this may also be partially true, since in a nonpausing population net spring rate differs from production for the spring period by a constant (the reciprocal of the number of days in the period). So far as winter and summer rates are concerned, the number of days in the production period is not the same for all birds and hence this criticism is less applicable.

Table 4 also presents the standard errors of estimate for the coefficients of total determination. These have been obtained directly from the latter coefficients in accordance with formula 49 of Ezekiel (*2*). Their magnitude varies from 12.9 to 21.4 eggs for the different combinations presented and their values indicate the degree of reliability to be placed on the multiple correlation values.

Table 5 presents the constants of the multiple regression equations from which estimation of either the 365-day or the biological laying-year production may be made. These were used for the calculation of the part determinations.

TABLE 5.—*Constants of the multiple regression equations, 1933 and 1934 series*

Series	Dependent variable	Age at first egg	Age at last egg	Winter rate	Spring rate	Summer rate	Free term
1933	365-day production	−0.52	0.34	2.25			−3.40
		−.48	.31		2.67		−39.22
		−.53	.31			1.92	45.23
	Biological laying-year production	−.74	.60	2.33			−102.15
		−.70	.57		2.66		−131.94
		−.75	.57			1.90	−46.06
1934	365-day production	−.42	.40	1.55			1.27
		−.55	.36		2.47		−41.61
		−.55	.44			1.68	−8.56
	Biological laying-year	−.64	.69	1.77			−123.08
		−.78	.66		2.40		−149.21
		−.76	.74			1.77	−127.79

RELATIVE IMPORTANCE OF THE INDEPENDENT VARIABLES

The determination of the relative influence of the independent variables on the dependent factor is a somewhat complex procedure. A number of methods are available for this purpose, but it may often happen that different interpretations are possible when different methods are used. When equal or proportional numbers of observations for various subclasses are available, an analysis of variance probably serves the purpose best. However, when such is not the case and when the making of certain assumptions necessary for such an analysis is not a justifiable procedure, other methods have to be used. Ezekiel (*2*) presents four such methods, of which three have been used for this study. The fourth, involving the calculation of coefficients of separate determination, does not give the net action of each of the independents on the dependent variable, and includes the interactions between the independents in the final values obtained. Furthermore, Ezekiel does not recommend the use of this method on a number of other grounds.

The three methods used here involve the determination of the coefficients of partial correlation, the coefficients of part correlation, and of the partial beta coefficients. The relative magnitude assigned to each of the independent variables affords a measurement of their importance in influencing the dependent factor.

The first of these coefficients is a measure long familiar to the workers in the field of inheritance of egg production. The squared coefficient of partial correlation measures the reduction due to the added factor in the variance remaining after the effects of the other independent factors have been accounted for. It is calculated in accordance with Ezekiel's formula 50, and the values obtained appear in table 6 under the heading "Reduction in unexplained variance."

TABLE 6.—*Relative importance of factors affecting the annual record, 1933 and 1934 series*

Dependent factor	Factors already considered	Independent factor	1933 series			1934 series		
			Reduction in unexplained variance	Coefficient of part determination	Partial beta squared	Reduction in unexplained variance	Coefficient of part determination	Partial beta squared
365-day production	Age at last egg; winter rate	Age at first egg	0.303	0.116	0.231	0.554	0.159	0.151
	Age at first egg; winter rate	Age at last egg	.282	.094	.195	.543	.315	.289
	Age at first egg; age at last egg	Winter rate	.331	.125	.243	.537	.132	.130
	Age at last egg; spring rate	Age at first egg	.405	.198	.197	.510	.240	.258
	Age at first egg; spring rate	Age at last egg	.362	.159	.162	.455	.226	.235
	Age at first egg; age at last egg	Spring rate	.633	.424	.458	.469	.298	.312
	Age at last egg; summer rate	Age at first egg	.354	.144	.240	.428	.190	.240
	Age at first egg, summer rate	Age at last egg	.230	.086	.162	.513	.282	.350
	Age at first egg; age at last egg	Summer rate	.412	.202	.319	.365	.129	.174
Biological laying year production	Age at last egg; winter rate	Age at first egg	.578	.341	.305	.775	.465	.185
	Age at first egg; winter rate	Age at last egg	.625	.417	.397	.841	.707	.154
	Age at first egg; age at last egg	Winter rate	.456	.193	.170	.701	.260	.089
	Age at last egg; spring rate	Age at first egg	.673	.444	.272	.530	.288	.275
	Age at first egg; spring rate	Age at last egg	.706	.523	.358	.662	.407	.416
	Age at first egg; age at last egg	Spring rate	.657	.466	.296	.181	.158	.156
	Age at last egg; summer rate	Age at first egg	.582	.336	.312	.585	.352	.260
	Age at first egg; summer rate	Age at last egg	.569	.375	.358	.751	.558	.523
	Age at first egg; age at last egg	Summer rate	.431	.224	.203	.378	.132	.102

The coefficient of part correlation differs from the first measure in that the adjustment for variation is made only in the dependent factor. The coefficient of partial correlation may be said to measure the relationship between the net variation of the independent factor from which the variation due to other independent factors is removed and the net variation of the dependent factor, similarly adjusted. The coefficient of part correlation, on the other hand, measures the relationship between the latter and the gross or unadjusted variation of the independent factor considered. It is calculated from formula 51 of Ezekiel, and the squared values of it appear in table 6 in the columns headed "Coefficient of part determination."

The third method used involves the partial beta coefficients, which represent the coefficients of the regression equations placed on a comparable basis with each other by expressing them not in the units in which they were originally stated but in terms of their respective standard deviations. To avoid negative values the squares of these coefficients rather than the coefficients themselves as determined by Ezekiel's formula 52 are presented in table 6.

The values obtained by these three methods do not always give the same order of magnitude for the different variables, but general trends can be deduced from examination of the order in which they appear for the different regression equations. Thus perusal of table 6 brings out the fact that when the biological laying year production is considered, age at last egg is the single factor of greatest importance. Except in one instance, that of the 1933 series partial correlation coefficient when summer rate is used as the measure of intensity, higher values are obtained for age at last egg than for age at first egg or any of the three rate measures used. Age at first egg undoubtedly seems to be the factor next in importance, the rate measures showing the lowest values throughout with a minor exception in the case of the 1933 series, where spring rate gives a somewhat higher value than does age at first egg.

When the 365-day production is considered the picture is not as clear-cut. Age at last egg still may be considered as the most important factor, although in a number of cases it appears to be somewhat less important than rate. Age at first egg gives uniformly the lowest values for the 1933 series, but shows greater influence on the egg record in the 1934 series.

So far as the relative value of the three measures of rate is concerned, it is hard to pass judgment on the basis of table 6 alone. However, comparison of the data from this table with those of table 4 definitely establishes the point that summer rate is the least adequate of the three measures. Spring rate seems somewhat superior to winter rate, although in a number of cases, particularly when 365-day production in the 1934 series is considered, winter rate accounts for a greater percentage of variance than does spring rate when age at first egg and age at last egg have been already accounted for.

In general it may be considered as established that in this nonpausing population the time factors are of greater importance than the rate factor. Of the factors considered age at last egg, measuring persistency, is the greatest single factor affecting the expression of the inherent ability of a bird to lay eggs. The significance of this is enhanced by the fact that Asmundson (*1*) using similar methods of analysis found that this observation also holds true for turkeys, when date of last egg is used as a measure of persistency.

CONCLUSIONS AND SUMMARY

A biometric analysis of the egg production records of two selected nonpausing population of Single-Comb White Leghorn pullets reveals that:

(1) 55.3 to 91.4 percent of the variance in annual egg record as measured by 365-day or by biological laying-year production can be accounted for by the variation in age at first egg, age at last egg, and winter or spring or summer and fall rate.

(2) The variance of these factors is to a considerable degree genetic in nature.

(3) Of the factors considered age at last egg is the most important single factor affecting egg production.

(4) Net summer and fall rate of production is not an adequate measure of intensity, the use of spring or winter rate giving more accurate estimates of the annual production.

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COMPARATIVE REACTION OF OAT VARIETIES IN THE SEEDLING AND MATURING STAGES TO PHYSIOLOGIC RACES OF PUCCINIA GRAMINIS AVENAE, AND THE DISTRIBUTION OF THESE RACES IN THE UNITED STATES [1]

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INTRODUCTION

In the course of studies carried out at the Minnesota Agricultural Experiment Station on the reaction of different varieties and hybrids of oats to the stem rust fungus, *Puccinia graminis avenae* Eriks. and Henn., the following questions arose: (1) Would the results obtained from inoculating seedling plants with specific physiologic races correspond with the results procured when plants of the same varieties approaching maturity were inoculated with identical races of the fungus? (2) With how many and with which particular physiologic races is the oat breeder likely to be confronted in the United States in any given area or in the country as a whole? Field practice might be considerably simplified and expedited if heterozygous and homozygous F_3 lines could be classified on the basis of seedling reaction in greenhouse tests; and the problem of developing resistant varieties might be facilitated if adequate knowledge were available regarding the prevalence and distribution of the different physiologic races of oat stem rust.

Results obtained by various workers in the past have, in the main, indicated essential agreement between seedling and adult-plant reaction to rust within the limits of a given crop variety, although a few notable exceptions have been reported. It was to investigate this point for each known physiologic race of *Puccinia graminis avenae,* tested on standard varieties and promising strains of oats, that the present study was undertaken. The work was greatly simplified by the use of purified cultures of all known physiologic races of oat stem rust, by the availability of some very important varieties and strains of oats commercially grown or in the process of development, and by the accumulation of rust-survey data over a period of many years.

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[2] The writers are indebted to Drs. E. C. Stakman and H. K. Hayes, of the Minnesota Agricultural Experiment Station, for their interest and assistance in this investigation; to Dr. W. L. Gordon, of the Dominion of Canada Rust Research Laboratory, and to Dr. R. U. Cotter, of the Division of Plant Disease Control, Bureau of Entomology and Plant Quarantine, U. S. Department of Agriculture, for uredial material of the several parasitic races of oat stem rust used in this study; to members of the Division of Plant Disease Control, Bureau of Entomology and Plant Quarantine, and others, too numerous to list by name, who have supplied rust specimens from various parts of the United States in the course of many years, and to Drs. E. C. Stakman, D. L. Bailey, A. W. Henry, J. M. Wallace, and R. U. Cotter for valuable help in identifying many of the stem rust collections forming part of the survey incorporated in this paper.

with stem rust. Similar results were obtained with crown rust. Durrell and Parker (*6*) found that oats in the seedling and heading stages reacted similarly to crown and stem rust in greenhouse and field tests. Stakman, Levine, and Bailey (*31*) stated that "as a result of previous work, it is safe to conclude that the reaction of seedlings to rust forms is a fairly accurate index of the reaction of older plants" where oat stem rust is concerned. It appears from their description of methods, that Mackie and Allen (*21*) might have noted a positive correlation between seedling and adult plant reaction, though no definite statement concerning this point was made by them.

The results obtained by Bailey (*2*) on the reactions of seedlings and adult plants of oats to stem rust indicate the existence of a close agreement in the material he used. Gordon (*8*) found that some 230 varieties and strains of oats of American and European origin were all highly susceptible in the seedling stage to race 6 of *Puccinia graminis avenae*, and that the seedlings of all but 1 of these varieties were about equally susceptible to race 4. In a field test these two rust races were similarly virulent on adult plants. Levine, Stakman, and Stanton (*20*) noted a general agreement in the reaction of certain varieties of oats, grown in uniform rust observation nurseries at various experiment stations during the 5-year period 1923–27, with the reaction of seedling plants grown in the greenhouse to physiologic races of stem rust isolated from these nurseries. Welsh (*40*) demonstrated that in all cases where resistant lines of oats were tested with races 1, 2, 3, 5, and 7 of *P. graminis avenae*, in the greenhouse and in the field, there was full agreement in the reaction of the seedlings with that of the maturing plants. According to Gordon (*9*), essentially the same results were obtained with seedling and adult plants of Joanette strain, when inoculated with identical physiologic races of stem rust of oats and cultured under comparable temperature conditions. Smith (*29*) noted essential agreement between seedling reaction to stem rust and crown rust of oats, grown under greenhouse conditions, and the reaction of adult plants of the same F_3 families when grown in the field, in the environment existing at University Farm, St. Paul, Minn.

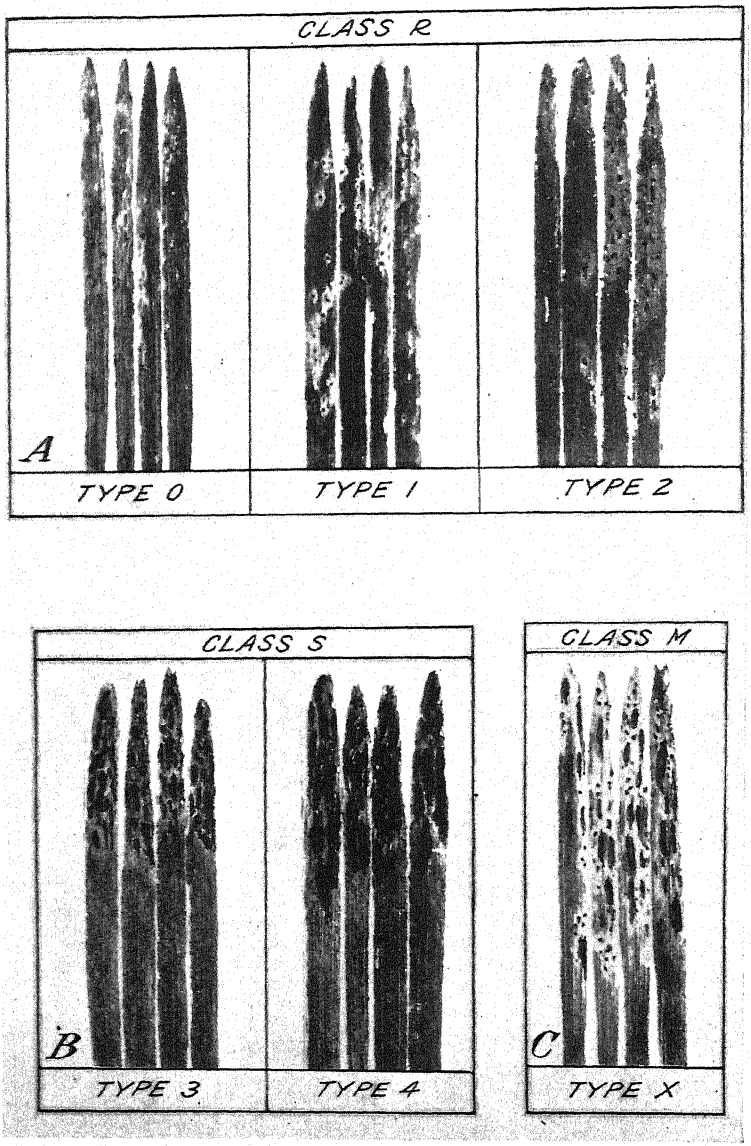
The foregoing review reveals a striking uniformity in the results obtained by various workers regarding the relative susceptibility to rust of oat plants at different stages in their development. In virtually all cases there appeared to be a harmonious agreement in the reaction of seedling and adult plants of oats to both stem and crown rust. No such correlation prevailed in every instance reported for wheat varieties, as was brought out in the preceding section.

MATERIALS AND METHODS

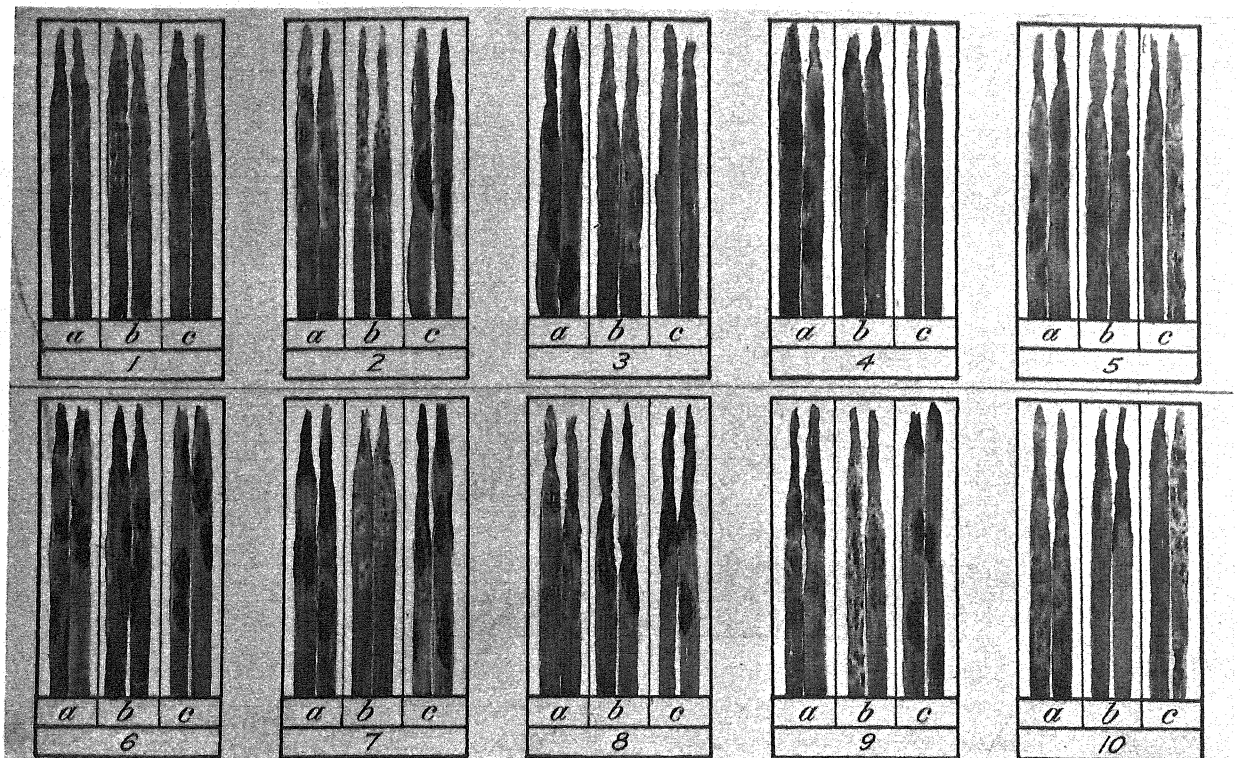
The oat varieties of the present study, tested both in the adult and seedling stages, were as follows: Gopher (C. I.[4] 2027), Victoria (C. I. 2401), Minnesota 742 (C. I. 2874)[5], Anthony (C. I. 2143), Minrus (C. I. 2144), Rainbow (C. I. 2345), and Richland (C. I. 787). These oat strains were used because of their importance in the breeding project of the Minnesota Agricultural Experiment Station or because of differential characteristics valuable in the identification of stem rust races. The varieties Gopher and Victoria always have been highly

[4] C. I. refers to accession number of the Division of Cereal Crops and Diseases (formerly Office of Cereal Investigations).

[5] Hybrid selection, Minnesota nursery stock no. H-22-220.



Reaction classes assigned to differential varieties, based on infection types produced by physiologic races of stem rust on seedling plants of oats: *A*, Resistant class, exemplified by type *0* (no infection at all or pronounced necrotic flecks), type 1 (minute uredia surrounded by distinct necrotic areas), and type 2 (small uredia embedded in hypersensitive areas ranging from slight necrosis to definite chlorosis); *B*, susceptible class, portrayed by type 3 (medium size uredia with some chlorosis but no necrosis), and type 4 (large and confluent uredia resulting in severe infection); *C*, mesothetic class, marked by type X (infection heterogeneous and rather ill-defined).



Reaction of seedling plants of three differential varieties of oats to 10 physiologic races of *Puccinia graminis avenae*. Arabic numerals denote key numbers of respective physiologic races. The differential varieties represented are designated as follows: (*a*), White Tartar, (*b*), Richland, (*c*), Sevnothree. (Compare illustrations with results given in table 1.)

susceptible to all known physiologic races of *Puccinia graminis avenae*. Anthony, Minrus, and Minnesota 742 react very much like their White Tartar (White Russian) parent to the various rust races. The stem rust reactions of Rainbow and Richland are virtually identical, and either may serve as a substitute differential host for the other.

Gopher, a pure-line selection from Sixty-Day, was described in detail by Stanton, Griffee, and Etheridge (*35*). Victoria was described by Murphy and Stanton (*23*) and again by Stanton and Murphy (*36*). Minnesota 742 is a strain that was obtained from crossing Minota×White Tartar by Black Mesdag; it is generally extremely resistant to loose and covered smut, and is at least moderately resistant to most parasitic races of stem rust. Minrus was described by Wilson and Arny (*42*) as Minnesota 693. Anthony and Rainbow were described by Stanton, Gaines, and Love (*34*). Richland was described by Burnett, Stanton, and Warburton (*4*).

In addition to the seven varieties just enumerated, six others were tested in the seedling stage to all known parasitic races of *Puccinia graminis avenae*, i. e., races 1 to 10, inclusive, and in the adult stage to race 6 alone. Fourteen more varieties were tested to all 10 races, but only in the seedling stage. A key for the identification of these rust races follows.

Analytical key for the identification of physiologic races of Puccinia graminis avenae on the basis of their pathogenicity on three selected differential varieties within the genus Avena

Reaction of differential hosts:	*Physiologic race (key no.)*
White Tartar resistant:	
Richland resistant:	
Sevnothree resistant	1
Sevnothree mesothetic	5
Sevnothree susceptible	2
Richland mesothetic	9
Richland susceptible:	
Sevnothree mesothetic	10
Sevnothree susceptible	8
White Tartar susceptible:	
Richland resistant:	
Sevnothree resistant	3
Sevnothree susceptible	7
Richland susceptible:	
Sevnothree resistant	4
Sevnothree susceptible	6

The various types of infection that the oat stem rust fungus is capable of producing under different circumstances are illustrated in plate 1. The parasitic behavior of the several rust races, cultured at a temperature fluctuating around 70° F., is recorded in table 1. The reactions of seedlings of three differential varieties of oats to each of the 10 physiologic races are depicted in plate 2.

TABLE 1.—*Reaction of differential varieties of Avena spp., in the seedling stage, to physiologic races of Puccinia graminis avenae, expressed in ranges and means of infection types*

Physiologic race (key no.)	Reaction of differential hosts [1]								
	White Tartar			Richland			Sevnothree		
	Range		Mean	Range		Mean	Range		Mean
	Minimum	Maximum		Minimum	Maximum		Minimum	Maximum	
1	2−	3n+	2+	0;	1++	1	1−	2++	1±
2	2=	3n+	2++	1−	2+	1+	4=	4+	4
3	3	4+	4−	1	2++	1	0;	1	1−
4	4−	4++	4	4−	4++	4+	0;	1+	1
5	1++	3n+	2++	1−	2+	1++	1c	X++	X±
6	4+	4++	4+	4+	4++	4+	4+	4++	4+
7	3	4+	4	0;	2+	2=	3	4+	4−
8	0;	2+	2	3	4+	4	3	4	3++
9	2=	3n+	2±	X=	4n+	X+	3++	4++	4
10	1	2+	2	3	4++	4	X−	X++	X+

[1] 0, practically immune (no infection whatever or pronounced necrotic flecks or lesions); 1, extremely resistant (minute uredia surrounded by solid necrotic areas); 2, moderately resistant (uredia small with hypersensitive areas varying from sharp necrosis to pronounced chlorosis); 3, moderately susceptible (medium-size uredia with slight chlorosis but no necrosis); 4, extremely susceptible (large confluent uredia resulting in very severe infection); X, characteristically mesothetic (infection heterogeneous and ill-defined). Plus and minus signs indicate a slightly greater or smaller amount of rust within a given infection type; the sign of equality denotes double minus. Necrotic flecks are designated by a semicolon; n=distinct necrosis; c=apparent chlorosis.

Races 1 to 5, inclusive, were originally described by Stakman, Levine, and Bailey (*31*), and later elaborated on by the last-named author (*2*). Further studies with these same races, as well as races 6 to 9, inclusive, were reported in detail by Gordon (*9*). Race 10 was first discovered and described by Cotter (*5*). The pathogenic behavior of all these races was summarized by Stakman et al. (*32*). A few of the races are widely distributed and of common occurrence, some are extremely virulent, others are relatively innocuous.

The procedure for testing the different oat varieties in the seedling stage was, except for some minor modifications, essentially the same as that described by Stakman, Levine, and Bailey (*31*). The method of testing the adult plants was as follows: Of the seven varieties previously listed, all but Richland were planted in three sets, sown 3 days apart. Each set consisted of thirty 4-inch pots, making 90 pots for each variety. After the pots had been steamed for half an hour at 12 pounds pressure, they were filled with soil steamed for 2½ hours. Three to five seeds of a given variety were sown in each pot. When the plants reached the early seedling stage, they were thinned to a single plant per pot and allowed to grow to a late heading stage.

All potted plants were set on benches in a greenhouse where for some time previous there had been no stem rust cultured. Here the plants grew under natural light conditions, which in the course of the experiment were unusually favorable. The atmospheric temperature in the greenhouse fluctuated between 67° and 80° F. The pots were at first placed close together; as the plants became larger, these pots were arranged in double rows, allowing a 6-inch space between pairs. When the plants attained a height of 8 inches, a cord lattice was made to hold them erect. When they were approximately 20 inches tall,

the cord support was removed and in its stead individual wire supports for each plant were substituted.

The plants were watered as frequently as necessary to prevent severe wilting, usually every 24 hours. The growth produced was somewhat more succulent and tender than that usually attained by plants grown in the field. What effect, if any, such succulence had on the reaction of the plants to the rust attack was not experimentally determined, except as manifested by the character of the rust development and the resultant infection types.

Eight days before a given set of adult plants was inoculated, seed of corresponding varieties was sown in 4-inch pots to provide seedlings for simultaneous inoculation with the respective physiologic races. At the time of inoculation, the adult plants were 30 to 40 inches tall and in the boot stage. There were one to five tillers per plant, all plants appearing vigorous and showing no indication of chlorosis or other weakness. Immediately preceding inoculation, the potted adult plants were placed in tall metal cylinders, which rested in pans containing water to a depth of 1½ to 2 inches. Each incubation chamber contained 20 plants of two separate oat varieties or strains.

The different sets of oat plants were inoculated seriatim with urediospores of a particular race in the following manner: The adult plants were sprayed with tap water and thoroughly brushed with rusted seedling plants of Victory oats severely infected by a known rust race that had been carefully tested for purity. The moist chambers then were covered with glass panes. Three days later the inoculated plants were removed from the incubators and placed on greenhouse benches. Seedling plants were inoculated at the same time and incubated for 2 days instead of 3. Incubation conditions were comparable for the different oat sets and growth stages. This method was used for all physiologic races except race 10, which was not available at the time. Subsequently, race 10 also was used to test the reaction of seedling and adult plants of the seven oat varieties under uniform cultural conditions. The variety Richland was tested as a unit to each of the first nine physiologic races, but was included with the other six varieties when their reaction to race 10 was determined. A special test of the reaction of adult plants to race 6 was made with six additional varieties, viz, Hajira (C. I. 1001), Iogold (C. I. 2329), Joanette strain (C. I. 2660), Sevnothree (C. I. 3251), Victory (C. I. 1145), and White Tartar (C. I. 551).

Rust notes were taken on each of the sets 17 days after their respective inoculations. At that time pustules on both seedlings and maturing plants had reached their optimum development. Although the infection varied with the different races on the several varieties, it was generally rather severe on the susceptible ones. The reaction of all varieties and strains, even when resistant, was clear-cut. Three reaction classes were recognized, namely, resistant, mesothetic, and susceptible. These were designated in the records by the capital letters R, M, and S, respectively.

As may be seen from plate 1, the resistant class embraces infection types 0, 1, and 2 (type 0 indicating immunity, type 1 high resistance, and type 2 moderate resistance); the mesothetic class includes but a single infection type, X, indicating a heterogeneous and ill-defined, somewhat intermediate reaction; and the susceptible class is represented by types 3 and 4 (type 3 standing for moderate and type 4

for complete susceptibility). In this study, rust observations on seedlings are recorded in terms of detailed infection types, whereas readings on adult plants are recorded in terms of general reaction classes. The respective symbols are used to denote each. Wherever necessary, plus and minus signs are used to show greater or smaller fluctuations within a norm.

RESULTS OF INVESTIGATION

The results obtained from the tests with seedlings and adult plants of the different oat varieties are summarized in tables 2 and 3 and illustrated in plates 3 to 8, inclusive. The occurrence of physiologic races of oat stem rust [6] and their relative prevalence in the United States are recorded in tables 4 and 5.

TABLE 2.—*Average infection produced by 10 physiologic races of Puccinia graminis avenae on seedlings of 27 varieties and strains of oats grown under greenhouse conditions and constituting four distinct reaction, or differential, groups*

Variety tested			Mean infection type produced by physiologic race (key no.)—									
Reaction group no.	Name	C. I. no.	1	2	3	4	5	6	7	8	9	10
1	Anthony	2143	2=	2−	4+	4+	2−	4−	4	2−	2−	2±
	Green Mountain	1892	2	2−	4	4+	2±	4	4−	2	2+	2
	Minnesota 742	2874	2=	2−	4	4+	1±	4−	4+	2−	1=	2−
	Minrus	2144	2−	2=	4+	4+	2−	4±	4±	2−	2±	2±
	White Tartar	551	2±	2−	4+	4+	2−	4±	4+	2−	2=	2±
2	Hajira	1001	1−	1−	1+	4	1	4±	2=	4	X±	4±
	Hawkeye	2464	2=	1±	2=	4−	2	4−	2	4−	X+	4+
	Iogold	2329	1	1−	1±	4+	1−	4+	1+	4+	X+	4+
	Iowa D67	2870	1−	1+	2=	4+	2±	4+	2+	4	X=	------
	Rainbow	2345	1−	1±	2−	4+	1	4±	1+	4±	X+	4=
3	Richland	787	1±	1+	1+	4±	1±	4±	1±	4±	X±	4−
	Joanette strain	2960	1±	4+	1−	1−	X	4+	4±	4+	4+	X
	Sevnothree	3251	1	4	1−	1±	X±	4±	4−	4=	4	X±
4	Alber	2766	4−	4+	4±	4+	4+	4−	4	4+	4+	4+
	Belar	2760	4	4−	4	4−	4−	4	4	4	4	4+
	Bond	2733	4±	4	4=	4+	4−	4+	4±	4+	4+	4
	Cassel	2911	4+	4+	4+	4+	4+	4+	4+	4+	4+	4+
	Cowra	2761	3+	4	4	4±	4+	4	4+	4=	4	4+
	Fulmer	2912	4=	4−	4	4	4+	4	4+	4	4	4+
	Glabrota	2630	3+	4	4	3+	4=	4	4	4	4	4+
	Gopher	2027	4=	4	4+	4+	4	4+	4	4±	4±	4
	Kareela	2774	4	4±	4	4−	4+	4+	4±	4+	4+	------
	Ruakura	2025	4+	4±	4+	4+	4+	4+	4	4+	4+	4+
	Sterisel	2891	4	4±	4	4+	4	4+	4	4	4+	4+
	Swedish Select	134	4+	4	4+	4+	4+	4+	4	4	4−	4
	Victoria	2401	4+	4±	4	4	4+	4+	4±	4+	4+	4+
	Victory	1145	4+	4±	4	4±	4+	4+	4+	4+	4+	4±

[6] In conformity with the decision of the Sixth International Botanical Congress, the designations "parasitic and/or physiologic race or races" are given preference in this paper over their former equivalents, "biologic and/or physiologic form or forms."

TABLE 3.—*Comparative reaction under greenhouse conditions of seedling and adult plants of seven varieties and strains of oats to 10 different physiologic races of stem rust (Puccinia graminis avenae)*

Physiologic race (key no.)	Inoculation results on varieties tested [1]													
	A				B						C			
	Rainbow		Richland		Anthony		Minnesota 742		Minrus		Gopher		Victoria	
	Seedlings	Adults	Seedlings	Adults	Seedlings	Adults	Seedlings	Adults	Seedlings	Adults	Seedlings	Adults	Seedlings	Adults
1	1	R	1	R	2	R	2	R	2	R	3	S	4	S
2	1	R	1	R	2	R	2	R	2	R	4	S	4	S
3	1	R	1	R	4	S	4	S	4	S	3	S	4	S
4	4	S	4	S	4	S	4	S	4	S	4	S	4	S
5	1	R	1	R	2	R	1	R	2	R	4	S	4	S
6	4	S	4	S	4	S	4	S	4	S	4	S	4	S
7	1	R	1	R	4	S	4	S	4	S	4	S	4	S
8	4	S	4	S	2	R	2	R	2	R	4	S	4	S
9	X	M	X	M	2	R	1	R	2	R	4	S	4	S
10	4	S	4	S	2	R	2	R	2	R	4	S	4	S

[1] The several varieties are grouped into three categories, A, B, and C, according to their reaction to the various physiologic races. For seedling plants the average infection type is given; for adult plants, the reaction class, as follows: R, resistant; S, susceptible; and M, mesothetic or intermediate in reaction.

TABLE 4.—*Summary of the distribution of physiologic races of Puccinia graminis avenae obtained from various parts of the United States during the 15-year period 1921–35, arranged according to standard geographic divisions and oat-growing areas*

Place of origin	Total of isolates	Distribution of physiologic race (key no.)—									
		1		2		5		7		10	
	Number	*Number*	*Percent*	*Number*	*Percent*	*Number*	*Percent*	*Number*	*Percent*	*Number*	*Percent*
Geographic divisions:											
Atlantic States	31	2	6.5	20	64.5	7	22.6	1	3.2	1	3.2
East North Central States	331	7	2.1	211	63.8	111	33.5	------	------	2	.6
West North Central States	965	12	1.2	703	72.9	249	25.8	1	.1	------	------
South Central States	375	8	2.1	260	69.3	107	28.6	------	------	------	------
Western States	43	1	2.3	30	69.8	12	27.9	------	------	------	------
Entire country	1,745	30	1.7	1,224	70.1	486	27.9	2	.1	3	.2
Oat-growing areas:											
Great Lakes:											
Ohio	84	------	------	56	66.7	28	33.3	------	------	------	------
Indiana	27	3	11.1	14	51.9	10	37.0	------	------	------	------
Illinois	60	1	1.7	39	65.0	20	33.3	------	------	------	------
Michigan	55	2	3.7	35	63.6	18	32.7	------	------	------	------
Wisconsin	105	1	1.0	67	63.8	35	33.3	------	------	2	1.9
Total	331	7	2.1	211	63.8	111	33.5	------	------	2	.6
Mississippi Valley:											
Minnesota	306	7	2.3	218	71.3	80	26.1	1	.3	------	------
Iowa	126	2	1.6	87	69.0	37	29.4	------	------	------	------
Missouri	49	------	------	38	77.6	11	22.4	------	------	------	------
Total	481	9	1.9	343	71.3	128	26.6	1	.2	------	------
Great Plains:											
North Dakota	145	2	1.4	93	64.1	50	34.5	------	------	------	------
South Dakota	119	------	------	86	72.3	33	27.7	------	------	------	------
Nebraska	77	------	------	59	76.6	18	23.4	------	------	------	------
Kansas	143	1	.7	122	85.3	20	14.0	------	------	------	------
Oklahoma	125	2	1.6	103	82.4	20	16.0	------	------	------	------
Texas	241	6	2.5	149	61.8	86	35.7	------	------	------	------
Total	850	11	1.3	612	72.0	227	26.7	------	------	------	------
Entire region	1,662	27	1.6	1,166	70.2	466	28.0	1	<.1	2	>.1

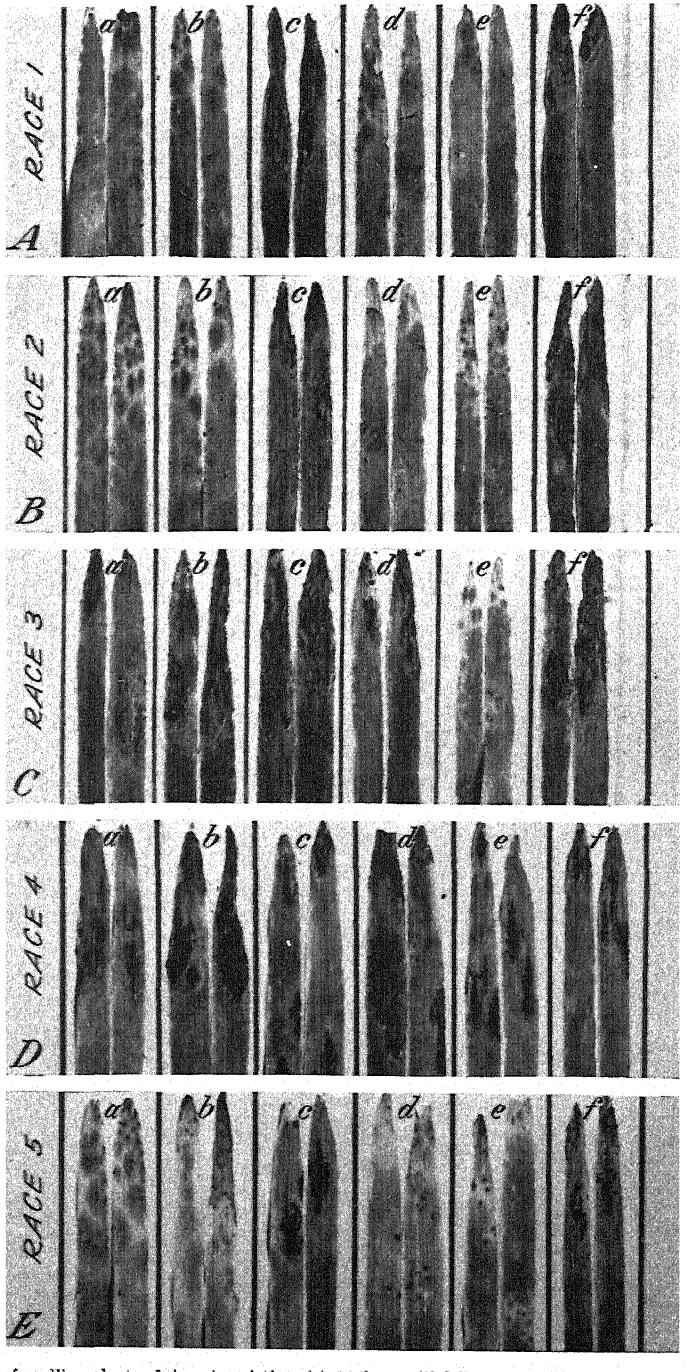
TABLE 5.—*Frequency of occurrence of physiologic races of Puccinia graminis avenae isolated from rusted field specimens obtained from different parts of the United States during the various years of the 15-year period 1921–35, inclusive*

Years	Total of isolates	Distribution of physiologic race (key no.)—									
		1		2		5		7		10	
	Number	*Number*	*Percent*	*Number*	*Percent*	*Number*	*Percent*	*Number*	*Percent*	*Number*	*Percent*
First 5 years:											
1921	12	5	41.7	7	58.3						
1922	12	3	25.0	6	50.0	3	25.0				
1923	13	7	53.8	5	38.5	1	7.7				
1924	51	1	2.0	30	58.8	20	39.2				
1925	29	2	6.9	27	93.1						
Total	117	18	15.4	75	64.1	24	20.5				
Second 5 years:											
1926	61	6	9.8	53	86.9	2	3.3				
1927	101			90	89.1	11	10.9				
1928	256			208	81.3	48	18.7				
1929	224	1	.4	155	69.2	68	30.4				
1930	144	1	.7	84	58.3	57	39.6			2	1.4
Total	786	8	1.0	590	75.1	186	23.7			2	.2
Third 5 years:											
1931	387	1	.3	260	67.2	126	32.5				
1932	108	1	.9	58	53.7	49	45.4				
1933	58	1	1.7	37	63.8	17	29.3	2	3.5	1	1.7
1934	130			99	76.2	31	23.8				
1935	159	1	.6	105	66.1	53	33.3				
Total	842	4	.5	559	66.4	276	32.8	2	.2	1	.1
Entire period	1,745	30	1.7	1,224	70.1	486	27.9	2	.1	3	.2

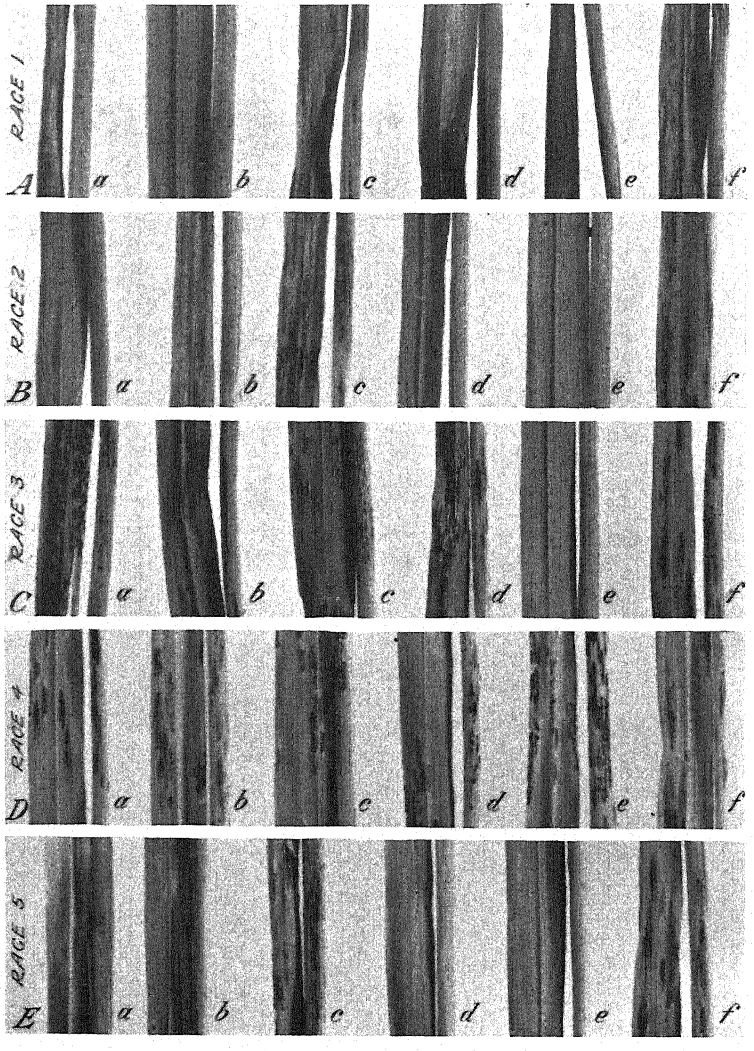
SEEDLING AND ADULT PLANT REACTION OF OAT VARIETIES

In table 2 are listed 27 varieties and strains of oats that were tested in the seedling stage, under fairly uniform greenhouse conditions, to all of the physiologic races of *Puccinia graminis avenae* known to date. It will be noted that on the basis of their reaction to the various rust races, these varieties were readily grouped into four categories. In the first three of these are included varieties possessing differential reaction characteristics that make possible the identification of physiologic races of the oat stem rust fungus. The varieties included in the fourth group show very little difference in their reactions, either with respect to each other or to the several physiologic races. Consequently, they are of no differential value in the determination of physiologic races; nor are they of parental value in breeding for stem rust resistance, although they may possess other desirable features.

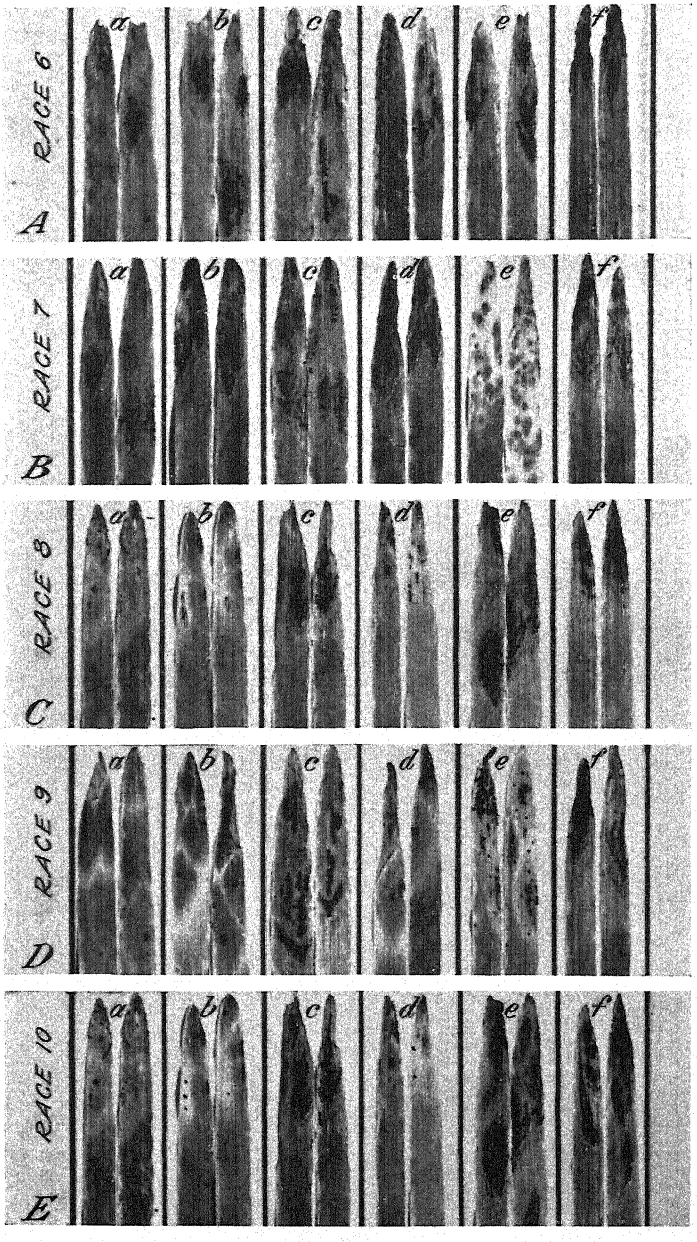
The three groups containing varieties with differential traits are listed in the order in which the standard differentials appear in the analytical key (p. 717) and infection record (table 1). The varieties within each group are arranged, for the sake of convenience, in alphabetical order. Incidentally, the last variety in each of these groups happens to be the standard differential host now used in the identification of physiologic races of *Puccinia graminis avenae*. The types of infection usually produced by these races on the differential varieties—White Tartar, Richland, and Sevnothree—are illustrated in plate 2. The origin of Sevnothree (strain 703) is described in detail by Bailey (*2*); reference to Richland has already been made; White Tartar is discussed by Levine, Stakman, and Stanton (*20*).



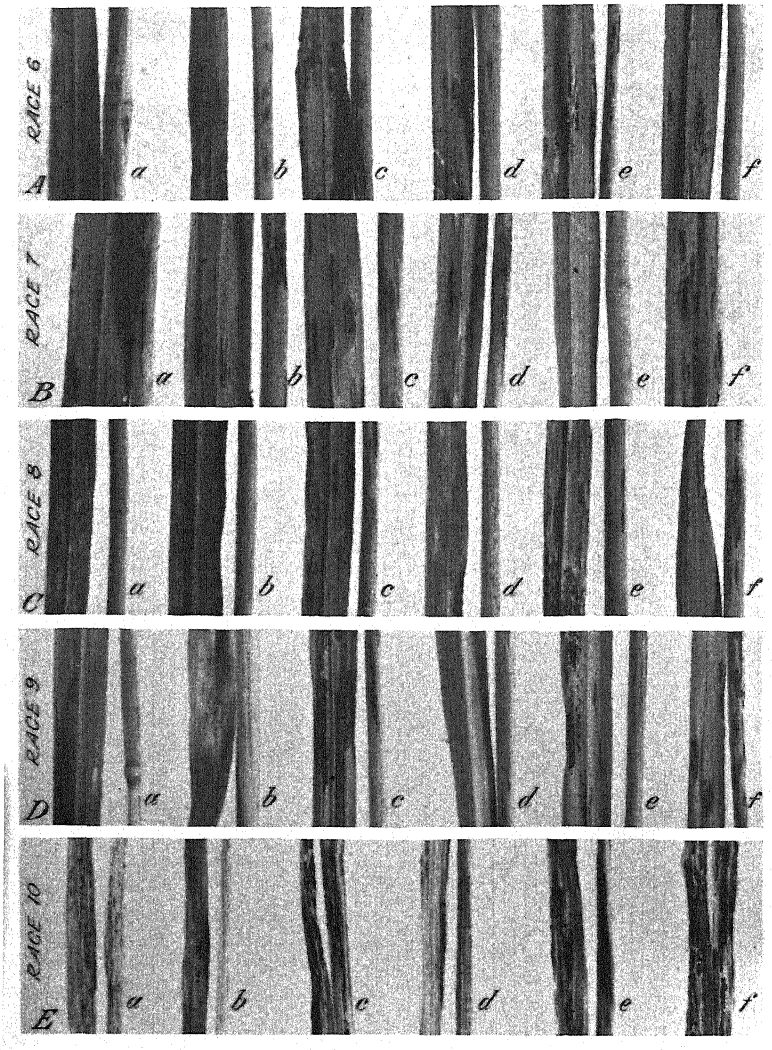
Reaction of seedling plants of six oat varieties—(*a*) Anthony, (*b*) Minn. 742, (*c*) Gopher, (*d*) Minrus, (*e*) Rainbow, and (*f*) Victoria—to five physiologic races of *Puccinia graminis avenae* (races 1 to 5, respectively). (Compare with reaction of adult plants of corresponding varieties, illustrated in plate 4.)



Reaction of adult plants of six oat varieties—(*a*) Anthony, (*b*) Minn. 742, (*c*) Gopher, (*d*) Minrus, (*e*) Rainbow, and (*f*) Victoria—to five physiologic races of *Puccinia graminis avenae* (races 1 to 5, respectively). (Compare with reaction of corresponding seedling plants, illustrated in plate 3.)



Reaction of seedling plants of six oat varieties—(*a*) Anthony, (*b*) Minn. 742, (*c*) Gopher, (*d*) Minrus, (*e*) Rainbow, and (*f*) Victoria—to five physiologic races of *Puccinia graminis avenae* (races 6 to 10, respectively). (Compare with reaction of adult plants of corresponding varieties, illustrated in plate 6.)



Reaction of adult plants of six oat varieties—(*a*) Anthony, (*b*) Minn. 742, (*c*) Gopher, (*d*) Minrus, (*e*) Rainbow, and (*f*) Victoria—to five physiologic races of *Puccinia graminis avenae* (races 6 to 10, respectively) (Compare with reaction of corresponding seedling plants, illustrated in plate 5.)

There are five varieties included in group 1. They either have side panicles or are the progeny of crosses in which a "side-oats" variety was one of the parents. They are all midseason or late in maturity. Each variety in this group is moderately resistant to six rust races (key nos. 1, 2, 5, 8, 9, and 10) and highly susceptible to the other four (key nos. 3, 4, 6, and 7). Group 2 consists of six varieties. All of these are common oats; they have yellow lemmas, and all but one are early maturing. They are, on the whole, very resistant to five races (key nos. 1, 2, 3, 5, and 7), mesothetic to one race (key no. 9), and very susceptible to four races (key nos. 4, 6, 8, and 10). In group 3 there are only two varieties. Both are pure-line selections from varieties possessing black lemmas and midseason maturity. They are extremely resistant to three parasitic races (key nos. 1, 3, and 4), typically mesothetic to two races (key nos. 5 and 10), and completely susceptible to five races (key nos. 2, 6, 7, 8, and 9).

The classification of the above oat groups is based on the infection types produced by the different stem rust races, cultured under favorable light intensities and at a prevailing atmospheric temperature of about 70° F. The reaction of the varieties in groups 1, 2, and 4 are but slightly affected by temperature and light variations, except in the case of race 9, where group 2 is concerned. Group 3, on the other hand, is very sensitive to changes in light and especially so to changes in temperature in the case of races 5 and 10. According to Gordon (*9*), Joanette strain loses its high resistance to races 1, 3, and 4 when cultured at high temperatures. Neither light nor temperature, however, seems to affect appreciably the susceptibility of Joanette strain and Sevnothree to the remaining five races.

The results summarized in table 3 and illustrated in plates 3 to 6, inclusive, reveal apparently complete agreement between the reaction of the seedlings of a given variety and that of the adult plants of the same variety. This correlation was not restricted to reaction to any one physiologic race, but held good for all of them; nor was it limited to any one oat variety, but prevailed for all seven varieties tested.

Plants of varieties on the seedlings of which a certain physiologic race produced an infection classified as type 1 or type 2 were invariably resistant in the adult stage also to that same race, and were designated by the symbol R. Though the distinction between infection types 1 and 2 was quite clear on seedling plants, corresponding adult plants could not be so readily differentiated according to infection type; therefore, the reaction class was used as the criterion for classification in the case of adult plants. The same held true to a great extent also in the case of susceptible reactions. Thus, plants on which infection types 3 and 4 developed in the seedling stage were always either moderately or wholly susceptible in the adult stage and were characterized by the symbol S. Although the infection produced by race 9 on adult plants of Rainbow and Richland was not typically heterogeneous, the reaction was more nearly mesothetic than otherwise, thus justifying its designation by the symbol M.

The varieties Rainbow and Richland reacted alike to all 10 rust races and were highly resistant in the adult as well as the seedling stage to 5 of these races. Anthony, Minnesota 742, and Minrus reacted very much the same to all races and were at least moderately resistant to six of them. Gopher and Victory were about equally

susceptible to all races. (Compare illustrations in plates 3 and 5 with those in plates 4 and 6, respectively, for corresponding results.)

Judging solely by the reaction of the 7 varieties just discussed, the 10 existing races could not be distinguished as so many units; instead, some of them could be combined in composite groups, as follows: Composite I, consisting of races 1, 2, and 5; composite II, embracing races 3 and 7; composite III, including races 4 and 6; and composite IV, consisting of races 8 and 10; with race 9 occupying an intermediate position between composites I and IV. This situation, obviously, is due to the fact that no representatives of oat group III, listed in table 2, were included among the seven varieties used in the maturative test. But essentially the same race combinations might be made even when Joanette strain or Sevnothree is used, as in the seedling experiment, if proper allowance is made for the somewhat erratic behavior of these varieties. Race 9 might be readily considered a counterpart of either race 8 or race 10, and thus a member of composite IV.

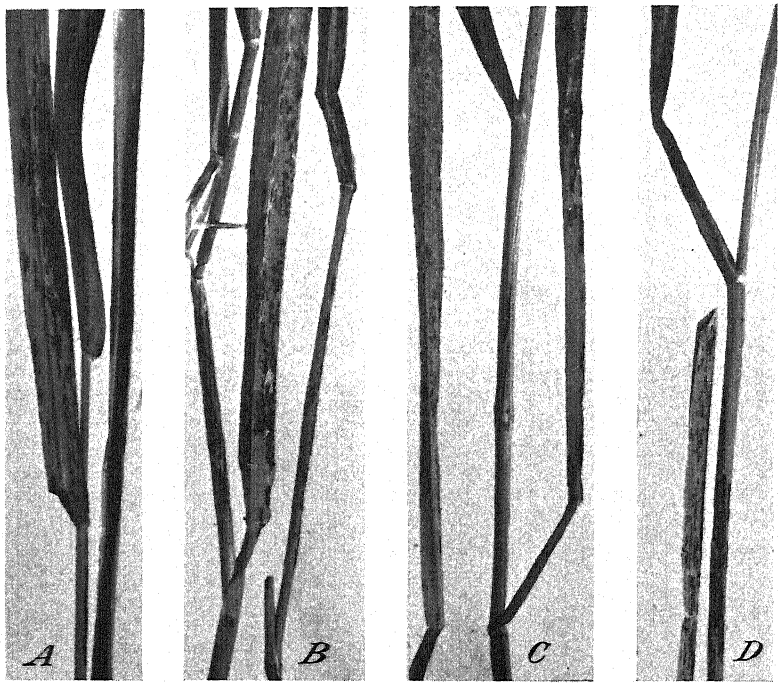
The results obtained in this study seem not to indicate any weakening in the resistance of the adult plants because of their succulent condition. While such a variety as Richland was highly susceptible in the adult stage to races 4, 6, 8, and 10 (pl. 7), it was extremely resistant to the other races. Considering expected reactions, the same was true of the other varieties. The reaction in each case depended on whether a physiologic race was virulent or innocuous on a particular oat variety. Race 6 is by far the most virulent of the lot,[7] causing severe infection on all varieties tested, seedlings and adult plants alike. The varieties depicted in plate 8 belong to all four oat groups, yet they are similarly affected by the rust of race 6. Races 4, 7, and 8 are next in order of virulence. These are followed by races 9 and 10, which in turn are followed by races 2 and 3. Then comes race 5, with race 1 last in order of virulence.

PREVALENCE AND DISTRIBUTION OF PHYSIOLOGIC RACES OF STEM RUST

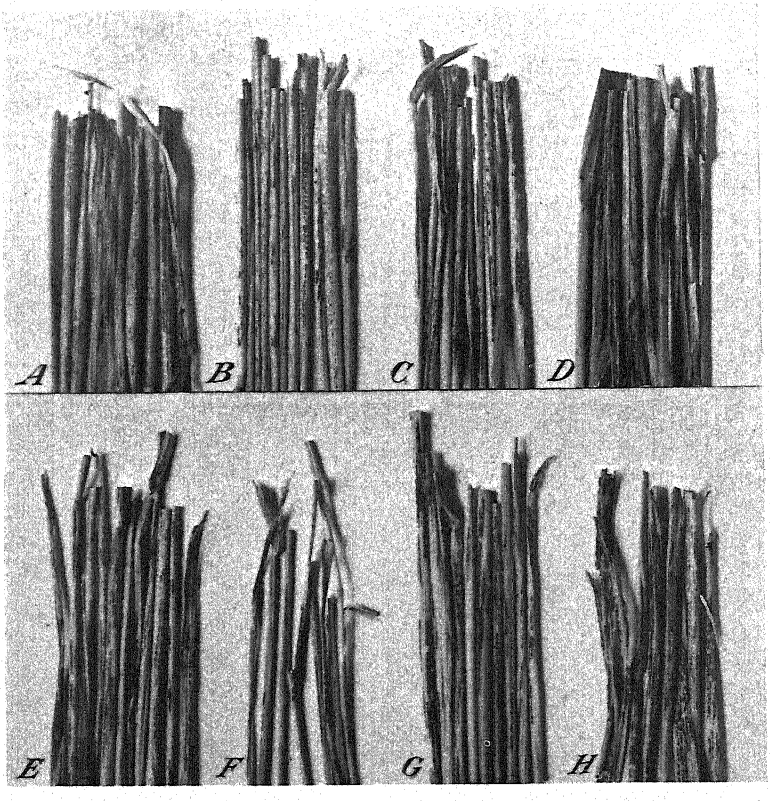
In view of the results obtained with regard to the relative susceptibility or resistance of oat plants at different stages of their development, the question of practical importance is "How many of the known races of *Puccinia graminis avenae*, and precisely which of them, exist in the United States, and what is their geographic distribution and frequency of occurrence?" A survey conducted for the past 15 years may afford an answer to this query.

An extensive study of the physiologic specialization of oat stem rust has been carried on at University Farm, St. Paul, Minn., since 1921. Although specimens from different parts of the world were identified, major attention was paid to field collections obtained from various sections of the United States. These were cultured in the greenhouse on the standard differential varieties, and their identity was determined with the aid of the analytical key (p. 717) and verified by means of the infection record (table 1). In all, five separate physiologic races have thus far been found to be present in variable frequency in this country. Since the identification was not carried out under strictly controlled environmental conditions, it is not impossible that other forms remained undetected.

[7] Since this manuscript was submitted for publication, Welsh (*41*) has reported securing a few oat lines from a cross of Hajira Strain × Joanette strain possessing greater resistance than either parent to several physiologic races of *Puccinia graminis avenae*, including race 6.



Relatively high and rather uniform susceptibility of adult plants of Richland oats to four physiologic races of *Puccinia graminis avenae*: *A*, Race 4; *B*, race 6; *C*, race 8; *D*, race 10. (Compare these infections with those produced by the same races on Richland seedlings, as illustrated in plate 2.)



Severe infection produced by physiologic race 6 of *Puccinia graminis avenae* on adult plants of eight varieties of oats, viz.: *A*, Anthony; *B*, Hajira; *C*, Iogold; *D*, Joanette strain; *E*, Richland; *F*, Sevnothree; *G*, Victory; *H*, White Tartar.

The races identified under the conditions of the experiments were key nos. 1, 2, 5, 7, and 10. Summaries of their distribution by geographic divisions and crop areas during the 15-year period under review, and their annual occurrence in the country as a whole are presented in tables 4 and 5, respectively. In the course of the entire 15 years from 1921 to 1935, altogether 1,745 isolations were identified. Only five of these, or 0.3 percent, constituted the total for races 7 and 10 combined.

The relative proportion of the number of times these two races were isolated to the total number of isolations made during the 15-year period is so insignificant, and the circumstances of their incidence of such a nature, that it appears doubtful whether either of them will soon, if ever, become a potent factor in the stem rust epidemics on oats in the United States. Race 7 thus far has been found only twice in this country: once, near rusted barberries at Presque Isle, Maine; and the second time, in the rust nursery at St. Paul, Minn. Race 10 was isolated three times: twice from specimens collected in the neighborhood of infected barberries at Jefferson, Wis.; and once, under like conditions, at Presque Isle, Maine.

Of the remaining three races, the least common for the period as a whole was race 1, which so far has been isolated but 30 times in the entire United States, constituting only 1.7 percent of all isolations made. Race 2 comprised 70.1 percent of the total and, on an average, was almost exactly two and one-half times as common as race 5, which constituted 27.9 percent of the total. There were no very great differences in the relative strength of these races in the several geographic divisions and crop areas, although considerable variation did occur between different States in the oat-growing region. Thus, Kansas furnished the highest proportion of race 2 (85.3 percent), while Indiana yielded the lowest (51.9 percent), taking the whole 15-year period into consideration.

A preponderant majority of the isolates (1,662 out of 1,745, or 95.24 percent) came from the oat-growing region. Of these, more than half (850) were obtained from the Great Plains area; the upper Mississippi Valley provided 481 isolates; and the Great Lakes area yielded 331. Minnesota furnished the highest number for a single State, while the fewest came from Indiana. Further details may be found in table 4.

It may be seen from table 5 that not only was there an annual fluctuation in the relative proportion of the three dominant races of *Puccinia graminis avenae* in the rust-survey territory of the United States but also there appeared to be a definite trend in the population relationships of these races, as determined by quinquennial computations. While throughout, except in 1 year, race 2 was invariably in the forefront, race 5, when present, was, with but two exceptions, in second place, and race 1, for the most part trailing behind, was at the head of the list once, in 1923. However, that was one of the years when the number of isolates obtained was very limited, and the apparent predominance of race 1 may not be significant.

In the first quinquennium (1921–25), race 1 occupied third position but was a close rival of race 5, and with 15.4 percent of the total number of isolates, seemingly was not a negligible factor in the oat stem rust epidemics of that period. However, it soon after all but disappeared, for in the second quinquennium (1926–30) it composed but

1.0 percent of the total, and in the third quinquennium (1931–35) it had dwindled to a mere 0.5 percent.

Race 2 follows a zigzag course, ranging from 38.5 to 93.1 percent of the respective annual totals. Considering the situation by 5-year periods, race 2 traced a rather gentle curve—64.1 percent in the first quinquennium, 75.1 percent in the second, and 66.4 percent in the third.

It is race 5 that shows a gradual and consistent quinquennial rise, from 20.5 to 23.7 to 32.8 percent of the respective 5-year totals. But this race, too, suffers from considerable annual variability, being entirely absent in 2 years and reaching 45.4 percent in at least 1 year.

It may be pertinent to observe at this juncture that, from a rather limited survey of the stem rust situation in Mexico, it appears that only races 2 and 5 are present in that country, and that up to this time they have occurred there in a 5:3 ratio. According to the results published by Bailey (*2*), including his data appearing in the report of the Dominion botanist (*3*), and by Gordon (*9*), the three races 1, 2, and 5 constituted 96.7 percent of the 1,342 isolations made by those authors from Canadian collections during the 10-year period 1921–30. The remaining 3.3 percent consisted of the other known races exclusive of race 10. Waterhouse (*39*) reports that in Australia races 1 and 2 together constituted over 88 percent of the 371 isolates of oat stem rust identified from 1925 to 1933, inclusive. Other races found there were 3, 6, and 7, the latter making up 8.1 percent of the total. A few isolations from New Zealand collections made by this authority yielded only races 1 "and/or" 2. From the results published by Verwoerd (*38*) it appears that races 3 and 7 occur in South Africa, while Stakman, Levine, and Bailey (*31*) had previously identified races 2 and 3 from material they received from that country. Tedin (*37*) noted the occurrence in Sweden of races 3, 4, 6, 7, and 8, but none of the prevalent American races. The present writers isolated races 2, 6, and 7 from uredial collections received from Palestine; and races 2, 5, and 7 from specimens obtained from New Zealand.

It is evident from the results recorded in this paper that the oat stem rust epidemics in the United States during the 15-year period 1921–35 were almost entirely due to the widespread distribution and common occurrence of races 2 and 5, the two races combined constituting 98 percent of the total number of isolates. This study has also brought out the fact that so far the physiologic specialization of *Puccinia graminis avenae* in this country has been rather definitely restricted.

SUMMARY AND CONCLUSIONS

Inoculations with the 10 known physiologic races of oat stem rust, *Puccinia graminis avenae*, were made under comparable and reasonably uniform greenhouse conditions on 27 varieties and strains of oats in the seedling stage; on 7 of these varieties in the adult stage, also with all 10 races; and on 6 more of the varieties and strains in the adult stage, with race 6 only.

Special care was taken to assure the purity of the physiologic races used. For this purpose periodic checks were made of the behavior of each race on the standard differential varieties grown under similar light intensities and fairly well-controlled temperatures. Assurance also was provided that the differential hosts themselves were free

from admixture. This was accomplished by the use of established pure rust races as biological reagents.

The differences in the reactions of the 27 oat varieties in the seedling stage made it possible to group them in 4 categories. In three of these were included the varieties that possess reaction characteristics typical of differential hosts, enabling their use as substitutes in the identification of physiologic races of *Puccinia graminis avenae*. The fourth group consisted of those varieties showing no discernible difference in their reactions, whether with respect to each other or to the different physiologic races.

There was a remarkably close agreement in the reactions of seedling and maturing plants of every one of the varieties and strains tested, regardless of parasitic race involved; in no case was a variety resistant or susceptible as a seedling or adult plant without a corresponding reaction in the other growth phase tested. It would seem from these results that seedling reaction is a reliable index of the reaction of adult oat plants to specific physiologic races of oat stem rust. It would be desirable, however, to investigate this problem further before drawing too general conclusions, since important exceptions, not considered within the limits of these experiments, may exist.

While during the 15-year period 1921–35, five different physiologic races (1, 2, 5, 7, and 10) of *Puccinia graminis avenae* have, at one time or another, been isolated from rusted oat material collected in various parts of the United States, only races 2 and 5 have played a significant part in the stem rust epidemics of this period. Race 1 was evidently of some consequence on certain occasions; but, on the whole, has been of trivial importance. Races 7 and 10, thus far, are of mere academic interest, so far as this country is concerned, but they may become more common in the future.

The restricted physiologic specialization of the oat stem rust fungus in the United States removes a serious impediment from the path of the plant breeder in his attempts to develop a desirable rust-resistant variety of oats. Adequate resistance to the dominant races 1, 2, and 5 is available in the varieties included in oat group 2, as listed in table 2. These varieties also are highly resistant to race 7. The varieties comprising oat group 1 are at least moderately resistant to the common races, as well as to race 10. To guard against the introduction or invasion of the more virulent races, it might be well to plan for the development of oat varieties with desirable agronomic characters that would resist their attack. Greenhouse experiments with seedling plants may facilitate and expedite the attainment of this goal.

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QUANTITATIVE DETERMINATION OF COPPER AND ESTIMATION OF OTHER TRACE ELEMENTS BY SPECTROGRAPHIC METHODS IN WIRE GRASSES FROM "SALT SICK" AND HEALTHY AREAS [1]

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INTRODUCTION

"Salt sick," a nutritional anemia, occurs among cattle, sheep, and swine, the feed of which is restricted to forage grown on certain types of sandy or peat soils. Earlier work of the Florida Agricultural Experiment Station demonstrated that the condition was caused by a deficiency of iron, or iron and copper, in the soils and forage (*2*).[3] Forage from salt sick areas was shown to be low in iron (*16*), and cattle grazing on these areas recovered when given an iron-copper supplement, as indicated by physical condition and hemoglobin content of whole blood (*15*). On certain soils, the affected animals responded erratically when iron was given as the sole supplement, but recoveries were quite uniform when a copper-iron supplement (1 : 50) was used.

A nutritional anemia having external and other symptoms similar to salt sick (*3*) has been reported as "bush sickness" or "skinnies" in New Zealand, "daising," "pining," "pine," and "vinquish" in southern Scotland, "nakuruitis" in Kenya Colony, East Africa, and "coasty disease" in King Island, Tasmania. An addition of cobalt to the ration was found to be helpful in certain parts of Australia and New Zealand (*1*, *21*). In Florida, iron and copper have been used successfully in correcting pure salt sick in field trials.

Cattlemen in Florida have long followed the practice of "burning the woods" (*4*) in order to remove old growth and give their animals the advantage of access to young vegetation in the late winter and early spring.

WORK OF OTHER INVESTIGATORS

The average iron content of wire grass, reported previously from this station (*16*), was 0.0177 percent in the dry matter of the grass (burned area) and 0.0168 percent (unburned area) from the deficient ranges, in contrast to 0.022 percent (burned) and 0.0193 percent (unburned) on healthy ranges. No analyses of grasses to determine their copper content have been reported from the Florida areas. The copper content of forage crops from other areas has been reported by a few investigators, as shown in table 1.

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[2] The authors are indebted to Drs. O. C. Bryan and R. B. Becker for collecting the grasses used in this study, and to Drs. R. B. Becker and W. M. Neal for determining the nutritional quality of the ranges.
[3] Reference is made by number (italic) to Literature Cited, p. 737.

TABLE 1.—*The copper content of forage crops reported by different investigators*

Forage crop	Method of analysis	Investigator	Copper per kilogram of dry matter
			Milligrams
Bluegrass	Xanthate	McHargue (*10*)	7.5
Kentucky bluegrass	Ferrocyanide	McHargue (*11*)	7.5
Alfalfa foliage			8.0
Red clover foliage			8.0
Kentucky bluegrass	Xanthate	McHargue (*12*)	14.0
Alfalfa hay:	Biazzo (modified)	Elvehjem and Hart (*5*)	9.1
First cutting (Wisconsin)			4.5
Second cutting (Wisconsin)			14.8
Colorado, sample 1			6.9
Colorado, sample 2			11.9
Bluegrass			8.3
Oat straw			3.8
Red clover hay			17.6
Rye straw			4.4
Soybean hay			8.2
Sweetclover hay			11.8
Timothy hay			2.2
Vetch hay			9.6
Wheat straw			2.1
Alfalfa hay	Xanthate	McHargue, Roy, and Pelphrey (*13*).	10.0
Alsike clover			6.0
Bluegrass hay			14.0
Orchard grass hay			5.0
Red clover hay			17.0
Redtop hay			4.0
Soybean hay			8.0
Sweetclover			9.0
Timothy hay			5.0
Wheat straw			3.0
Lecksucht area hay, average	Biazzo	Sjollema (*19*)	2.0–3.0
Lecksucht area hay, one case			3.0–5.0
Do			1.0
Normal hay, average			6.0–12.0
Normal hay, one case			5.0
Normal grass on pasture			20.0–30.0

METHODS OF INVESTIGATION

Samples of wire grass (*Aristida* sp.) were collected simultaneously with samples of soil (*3*) by plucking from areas on which cattle became salt sick, and on healthy areas from both burned and unburned ranges within a 2 months' period. The wire grass samples were analyzed spectrographically for quantitative differences in mineral content. The grass was dried at about 70° C. and stored in sealed glass jars. The samples selected for analyses were obtained from extreme salt sick, marginal,[4] and healthy areas.

All glass, platinum, and silica apparatus and equipment used were leached with hot 1 : 1 hydrochloric acid and rinsed several times with water obtained by triple-distillation through an acid-leached all-glass still. Spectrographic examination showed that this water contained less than one-half part per million of copper (*17*).

In earlier work (*16, footnote p. 251*) the senior author found that grinding wire grass in a Wiley mill increased its iron content by 0.0032 percent. To avoid contamination with metals, therefore, the grasses were broken by hand, placed in platinum or silica dishes, and dried at 100° C. After drying, the grasses were ashed for 24 to 48 hours in a muffle furnace at a temperature not exceeding 450°. The ash was then homogenized in an agate mortar and analyzed spectrographically.

[4] On marginal areas cattle do not develop salt sick, but affected cattle pastured on these areas do not recover.

QUANTITATIVE SPECTROGRAPHIC DETERMINATION OF COPPER

Many workers have reported quantitative spectrographic methods for the determination of copper in various materials. Gerlach and his coworkers (*7*, *8*, *18*) determined copper in biological materials with silver or cobalt as the internal standard; in this study cadmium was used.

To an accurately weighed portion of the ash of the sample a measured volume of a cadmium solution was added. This mixture was dried, carefully homogenized in an agate mortar, and small portions spectrographed on a Littrow spectrograph. Selected and tested purified graphite electrodes were used. In this connection, it should be noted that the best grade of graphite electrodes on the market frequently contains some copper, which may produce erratic results unless the electrodes are tested spectrographically prior to using, as was done in this study. Satisfactory and less expensive electrodes may be prepared by the purification methods of Standen and Kovach (*20*).

Great care was exercised at all times to avoid contamination. The solution of cadmium is known to contain a small proportion of copper, but the quantity thus added was negligible and was found not to affect the results.

Quintuplicate analyses of each sample were made. A nonrecording microphotometer was employed for measuring the ratio of the line densities. The probable error of the method is less than 10 percent of the mean.

RESULTS

The analyses for copper in the wire grasses from both healthy and salt sick areas that had been burned, or left unburned, are given in table 2. This table also presents the percentage of total ash on the dry-matter basis. The copper content of the wire grass samples from the salt sick areas ranged from 4.7 to 10 mg per kilogram of dry matter; those from the healthy areas ranged from 5.5 to 10.1 mg.

TABLE 2.—*Ash and copper content of wire grasses from salt sick and healthy (burned and unburned) areas as determined by spectrographic method*

Area, treatment, and sample no.	Ash (dry basis)	Copper per kilogram of dry matter [1]	Area, treatment, and sample no.	Ash (dry basis)	Copper per kilogram of dry matter [1]
Salt sick area, unburned:	*Percent*	*Milligrams*	Healthy area, burned, marginal: [2]	*Percent*	*Milligrams*
223	2.6	6.1±0.6	234	3.4	8.3± .8
243	3.1	7.6± .8	245	2.8	6.3± .6
251	2.9	5.4± .5	249	2.8	7.3± .7
261	2.6	5.3± .5	292	2.8	5.5± .5
262	2.6	4.8± .5			
263	2.7	4.7± .5	Average		6.8
265	2.9	7.4± .7			
275	2.6	4.8± .5	Healthy area, unburned:		
285	3.1	10.0±1.0	230	3.7	9.6±1.0
Average		6.2	232	3.9	7.5± .7
			235	6.2	10.1±1.0
Salt sick area, burned:			239	3.8	8.0± .8
237	2.5	6.6± .7	270	3.4	8.6± .9
241	3.0	8.0± .8	277	2.8	6.6± .7
258	3.3	9.3± .9	283	3.0	5.9± .6
Average		8.0	Average		8.0

[1] Mean of 5 determinations.

[2] This area not sufficiently supplied with available nutrients to be classed as strictly healthy (corrective), yet not so low as to be classed as salt sick.

DISCUSSION

A comparison of the copper content of samples of wire grasses collected from ranges on which cattle showed severe symptoms of salt sick, and those from ranges on which the animals were always healthy, failed to show any appreciable difference (table 2).

Neal and Becker (*16*), however, found that in the grasses from healthy areas the content of iron, calcium, magnesium, and phosphorus was higher than in the grasses from affected areas. An examination by Bryan and Becker (*3*) of the soil from both areas showed that the surface soils of the healthy ranges contained approximately 10 times as much iron, 5 times as much calcium and phosphorus, and twice as much copper as did those from the salt sick areas. These investigators report that "cattle will develop 'salt sick' on soils with 0.036 percent iron and 3.85 p. p. m. of copper, while they remain healthy upon soils with 0.42 percent of iron and 8 p. p. m. of copper."

The average values for copper in the grasses analyzed in the present work varied from 6.2 to 8 p. p. m. in salt sick and 6.8 to 8 p. p. m. in healthy areas, which indicates that the content of this element is not proportional in the vegetation and in the soil.

Since the grasses used in this work were obtained from extreme salt sick and healthy areas, the comparison of the copper content of these grasses indicates that copper deficiency per se is not the main etiological factor of salt sick although iron supplemented with copper was found to be effective in treating this condition in cattle. In the Netherlands, however, Sjollema (*19*) found an increase of about 5 p. p. m. in the copper content of hay from healthy areas as compared with hay from "Lecksucht" areas.

SPECTROGRAPHIC ESTIMATION OF OTHER TRACE ELEMENTS

Since no significant differences were found in the copper content of the wire grasses, it was decided to examine the ash samples for other trace elements. The procedure was designed to permit an estimation of the proportions of certain elements present in magnitudes detectable by spectrography on the original ash sample.

In this work no effort was made to increase the proportions of the elements in the samples by precipitation or any other chemical method. By concentration methods it probably would have been possible to detect the presence of some elements which are reported as "not detected;" but the fact that an element is found to be present in a very minute quantity is of less practical importance than a knowledge of its proportion in the sample. Accordingly, it seemed best to base this study on proportions of the elements detectable spectrographically in the ash of the sample.

A small proportion of the homogenized ash was volatilized in a 220-volt arc, a current of 9 to 10 amperes being used. Specially purified graphite electrodes were employed; repeated spectra of the graphite electrodes were made to insure a control of electrode impurities. In taking the spectrum of the sample, the arc was maintained until the sample was completely volatilized. Incomplete volatilization permits fractionation, which might involve a retention of the higher boiling elements in the residue and this vitiate the estimates of the amounts of the elements present.

A Littrow spectrograph with linear dispersion of about 30 inches between 2,250 A and 5,500 A was used. Two prisms were employed

with this instrument—a glass prism for lines of wave length greater than 3,800 A and a quartz prism for shorter wave lengths. To make use of the sensitiveness of the 2,138 A zinc line, a quartz Cornu type spectrograph with linear dispersion of about 9 inches between 2,100 A, and 8,000 A was employed for the zinc determinations.

The estimations were made by a modification of the comparison method used by Nitchie (*14*). Standard powders containing known percentages of the 24 elements included in the analysis were spectrographed in juxtaposition with spectrograms of the ash samples. The percentages of the elements present were then estimated by comparing visually the intensities of the spectral lines in the sample with corresponding lines in the standards. Duplicate determinations were made on each sample.

The data thus obtained are not intended as precision determinations, but are indicative of the "order of magnitude" of the proportions of the elements present. To avoid misunderstanding as to precision, and also to retain a legitimate basis for comparison, the data are presented in "range" form. For example, 1–10 recorded in table 3 should be read: The amount of the element in the sample lies between 1 and 10 mg per kilogram of dry matter.

The approximate sensitivity of the method is: For chromium, cobalt, nickel, and silver, between 0.0001 and 0.001 percent; for aluminum, barium, lead, manganese, molybdenum, strontium, tin, titanium, vanadium, yttrium, and zinc, between 0.001 and 0.01 percent; for antimony, boron, bismuth, cadmium, lanthanum, thallium, and zirconium, between 0.01 and 0.1 percent; and for arsenic and lithium between 0.1 and 1 percent.

The term "trace" signifies the smallest detectable proportion of the element in the ash This value converted to the dry-matter basis is almost negligible, but this does not alter the fact that the element was detected in the ash

RESULTS

The spectrographic estimations of the trace elements in the same wire grass samples that were used for the copper determinations are shown in table 3. Aluminum, barium, boron, lead, manganese, strontium, titanium, and zinc were detected in all the ash samples, in addition to copper

Of the grasses from the unburned salt sick area, chromium and silver were detected in traces in eight of the nine ash samples, and molybdenum in five. All the elements mentioned above except molybdenum were present in grasses from the burned salt sick and from the burned marginal areas Molybdenum was detected in two of the three ash samples from the burned affected area, and in two of the four samples from the burned marginal area

Chromium was detected in all the ash samples from the unburned healthy areas, in addition to the 10 elements present in all the grass samples from the burned areas Molybdenum and nickel were detected in three of the seven ash samples in this group

Antimony, arsenic, beryllium, bismuth, cadmium, cobalt, lanthanum, thallium, tin, vanadium, yttrium, and zirconium were not detected in any of the ash samples from these areas Nickel was detected only in the grasses from the healthy unburned area, and in one sample from the unburned salt sick area

TABLE 3.—*Spectrographic estimation of trace elements in wire grasses from salt sick and healthy areas expressed in milligrams per kilogram of dry matter* [1]

[Analyses made on ash, but for convenience converted to dry-matter basis. Duplicate analyses made]

Sample no.	Aluminum	Barium	Boron	Chromium	Lead	Manganese	Molybdenum	Nickel	Silver	Strontium	Titanium	Zinc
	Mg	*Mg*	*Mg*	*Mg*	*Mg*	*Mg*	*Mg*	*Mg*	*Mg*	*Mg*	*Mg*	*Mg*
Salt sick area, unburned:												
223	20–80	1–10	10–30	Tr	1–10	20–80	ND	ND	ND	<0 1	1–10	2–20
243	2–20	<0. 1	2–20	Tr	1–10	10–30	Tr	ND	<0. 1	<. 1	0. 1–1	30–100
251	10–30	. 1–1	2–20	Tr	0. 1–1	10–30	ND	ND	Tr	<. 1	. 1–1	10–30
261	20–80	1–10	2–20	Tr	. 1–1	20–80	ND	ND	Tr	<. 1	<0. 1	20–80
262	10–30	1–10	2–20	Tr	. 1–1	2–20	<0. 1	ND	Tr	<. 1	. 1–1	10–30
263	10–30	<. 1	10–30	ND	. 1–1	2–20	<. 1	ND	Tr	<. 1	<. 1	20–80
265	10–30	. 1–1	2–20	Tr	1–10	1–10	Tr	Tr	<. 1	<. 1	<. 1	20–80
275	2–20	. 1–1	1–10	Tr	. 1–1	10–30	Tr	ND	Tr	<. 1	<. 1	20–80
285	10–30	. 1–1	2–20	Tr	. 1–1	30–100	ND	ND	Tr	<. 1	<. 1	30–100
Salt sick area, burned:												
237	10–30	1–10	2–20	Tr	. 1–1	20–80	0. 1–1	ND	Tr	<. 1	. 1–1	10–30
241	20–80	1–10	2–20	Tr	. 1–1	30–100	<. 1	ND	Tr	<. 1	1–10	10–30
258	1–10	<. 1	2–20	Tr	. 1–1	1–10	ND	ND	Tr	<. 1	<. 1	30–100
Healthy area, burned, marginal:												
234	30–100	. 1–1	2–20	<0. 1	1–10	10–30	ND	ND	Tr	<. 1	1–10	1–10
245	20–80	1–10	2–20	<. 1	. 1–1	>60	. 1–1	ND	Tr	<. 1	. 1–1	20–80
249	20–80	1–10	2–20	<. 1	. 1–1	10–30	. 1–1	ND	Tr	<. 1	. 1–1	10–30
292	10–30	. 1–1	2–20	Tr	. 1–1	20–80	ND	ND	Tr	<. 1	<. 1	10–30
Healthy area, unburned:												
230	15–50	1–10	2–20	Tr	1–10	15–50	ND	ND	Tr	<. 1	. 1–1	30–100
232	30–100	1–10	2–20	Tr	1–10	10–30	ND	Tr	Tr	. 1–1	. 1–1	1–10
235	>60	20–80	10–30	<. 1	1–10	>60	ND	0. 1–1	Tr	<. 1	2–20	1–10
239	30–100	2–20	2–20	Tr	. 1–1	15–50	1–10	ND	Tr	<. 1	1–10	15–50
270	15–40	. 1–1	10–30	Tr	1–10	1–10	ND	ND	Tr	<. 1	1–10	30–100
277	10–30	1–10	2–20	Tr	. 1–1	20–80	Tr	Tr	<. 1	<. 1	<. 1	20–80
283	2–20	10–30	2–20	Tr	<. 1	10–30	1–10	ND	Tr	<. 1	Tr	10–30

[1] ND denotes not detected. Tr denotes trace (see text for discussion). Antimony, arsenic, beryllium, bismuth, cadmium, cobalt, lanthanum, thallium, tin, vanadium, yttrium, and zirconium were not detected in any of the ash samples.

DISCUSSION

A comparison of the values for the trace elements in the wire grasses from healthy and affected areas did not show any appreciable differences

Certain elements were detected in all the ash samples; hence, the results for these elements are at least consistent Other elements were detected irregularly, which indicates that the ash may have been contaminated

A high-titanium content in green parts of certain plants has been reported by Geilmann (*6*) and Headden (*9*), and unpublished work at this laboratory has confirmed this finding In the present study, titanium was detected in all the ash samples, but the proportions were low as compared with those reported by Geilmann and Headden.

Recently, workers at this station have found that cobalt is a limiting factor in one type of salt sick known specifically as "hill sick." The estimation of trace elements failed to show the presence of cobalt in any of the grass samples (table 3), yet this element is known to be beneficial to animals on certain areas. It should be pointed out that some of the elements reported in this study as not detected may have been present, but in amounts too small to be determined by the method used

The results obtained from this study suggest that salt sick may be the result of a combination of conditions which give rise to similar external symptoms. The ratio of the various elements involved may

be as important as the absolute amounts. Possibly salt sick may occur on grasses in which the iron content is high and the copper content low, or vice versa; or the ratio of iron or copper to some other element or elements may be involved.

Another factor that should be considered is the mineral content of the drinking water. In some water holes where an iron-bearing clay forms the substrate, the water is known to have a high content of iron and probably of other elements.

SUMMARY

A comparison of the results of spectrographic determination of the copper content of wire grasses from "salt sick", marginal, and healthy areas failed to disclose any significant differences.

The spectrographic estimation of other trace elements also showed no significant differences.

The following elements were detected in all the wire grass samples from the different areas: Aluminum, barium, boron, copper, lead, manganese, strontium, titanium, and zinc. The following elements were not detected in any of the samples: Antimony, arsenic, beryllium, bismuth, cadmium, cobalt, lanthanum, thallium, tin, vanadium, yttrium, and zirconium. Chromium, molybdenum, silver, and nickel were detected in the samples, but not consistently.

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FACTORS INFLUENCING THE DEVELOPMENT OF SOGGY BREAK-DOWN IN APPLES[1]

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INTRODUCTION

The influence of storage temperature on soggy break-down[2] in certain apple varieties is generally recognized, but the relation of other etiologic factors to this disease is not so clearly understood. For example, it has been assumed that delayed storage is essential to the development of soggy break-down, and that late picking favors its occurrence. The effect of these and other causal agencies has not been demonstrated conclusively. Among other factors which have been considered as favorable or essential to the development of soggy break-down are inadequate ventilation of containers and storage rooms, a prolonged storage period, temperatures lower than 32° F., variations in fertilizer treatments, and climatic conditions. The effect of these and certain other factors on soggy break-down is considered in this paper.

The importance of storage temperature in the control of soggy breakdown has been emphasized in earlier publications (*11*, *15*, *17*).[3] This paper presents further evidence to show the relation of storage temperature to the disease in the varieties that were previously listed as susceptible and in other varieties for which little or no information is on record. The relative importance of other storage and prestorage factors as judged by the results of more than 11 years' work is indicated.

REVIEW OF LITERATURE

Investigators have emphasized the importance of immediate storage in the control of the soft scald type of soggy break-down in the Jonathan apple (*1*, *3*, *6*, *18*, *19*). In an earlier paper (*13*) the writers indicated that delaying storage for 1 week, as compared with immediate storage or storage after 2 and 3 weeks' delay, usually increased susceptibility in this variety. It should be noted, however, that immediate storage did not invariably prevent the disease, and that delays of 2 and 3 weeks gave best control. On the other hand, delaying the storage of Grimes Golden and Golden Delicious has usually resulted in greater susceptibility to soggy break-down (*10*, *15*). Harding found that cold-storing Grimes Golden while it is in an active state of respiration produces a high degree of susceptibility (*4*), and that it reaches its highest respiratory activity between 3 and 5 weeks at 50° F.

Harley and Fisher (*6*) concluded that a high concentration of carbon dioxide in Jonathan apple tissues is not a contributory cause of soggy break-down; Brooks and Harley (*2*) found that short exposures to

[1] Received for publication Jan. 15, 1937; issued December 1937. Journal Paper No. J432 of the Iowa Agricultural Experiment Station; project No. 281.

[2] The two types of low-temperature disease designated in the literature as "soggy break-down" and "soft scald" are considered by the writers to be the same, and in this paper will be included under the term "soggy break-down." The reasons for this classification have been given (*11*, *17*).[3]

[3] Reference is made by number (italic) to Literature Cited, p. 762.

this gas and high temperature reduced the tendency toward the disease. Gerhardt and Ezell (*3*), working with Jonathan at Wanatchee, Wash., found that immediate storage at 32° F., when preceded by a 24-hour exposure to carbon dioxide, gave approximately as good control as ordinary storage at 36°. With delayed storage, however, carbon dioxide exposures gave less consistent results.

RELATION OF STORAGE TEMPERATURE TO SOGGY BREAK-DOWN

Although results of storage experiments of a rather comprehensive nature, showing that temperature is of primary importance in the control of soggy break-down in Grimes Golden, have been published (*11, 15, 17*), information concerning the effects of storage temperature on other varieties is limited. Some results for Jonathan, Wealthy, and Golden Delicious are on record (*9, 10, 14, 15*), but so far as the writers can determine, no results have been published for Northwestern Greening and Winter Banana.

STUDIES WITH JONATHAN

TABLE 1.—*Percentage of soggy break-down occurring in Jonathan apples during storage at different temperatures for three and for five seasons*

RESULTS DURING 3 SEASONS [1]

Temperature (° F.)	Delay before storing	Break-down in— [2]		
		1924–25	1926–27	1928–29
	Weeks	*Percent*	*Percent*	*Percent*
30	0	42.3	14.1	8.2
	1	18.9	6.9	67.6
	2	10.3	2.9	21.7
	3	0	1.0	15.2
32	0	6.9	0	1.8
	1	.6	0	19.1
	2	.6	0	16.2
	3	0	0	.7
34	0	0	0	0
	1	0	0	1.9
	2	0	0	5.1
	3	0	0	0
36	0	0	0	0
	1	0	0	.8
	2	0	0	.9
	3	0	0	0
Common storage	0	0	0	0
	1	0	0	0
	2	0	0	0
	3	0	0	0

RESULTS DURING 5 SEASONS [3]

Temperature (° F.)	Delay before storing	Break-down in— [4]				
		1929–30	1930–31	1931–32	1932–33	1933–34
	Days	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
30	0	76.2	8.7	0.0	11.2	0.7
	10	28.2	0	12.2	3.5	16.0
	20	1.4	0	0	0	0
36	0	0	0	0	0	0
	10	0	0	0	0	0
	20	0	0	0	0	0

[1] Fruit examined the latter part of January.

[2] Picking dates: 1924. October 7; 1926 and 1928, October 2. Source of Fruit: 1924, State orchard, western Iowa; 1926 and 1928, Apple Grove orchards, central Iowa. Number of boxes per test: 1 to 4. The same investigation was carried out in the seasons of 1925 and 1927. No soggy break-down occurred in these 2 years.

[3] Fruit examined in February 1929–30 and 1931–32 and in March 1930–31, 1932–33, and 1933–34.

[4] Picking dates: 1929 and 1931, October 2; 1930, Oct. 6; 1932, September 30. Source of fruit: 1929, 1930, and 1931, Apple Grove orchards, central Iowa; 1932 and 1933, Worth orchard, western Iowa. Number of boxes per test: 1 to 4. In 1934 there was no crop because of severe drought.

The effect of various storage temperatures on the development of soggy break-down in Jonathan is shown in table 1. These results show that break-down developed more severely at 30° F. than at 32°, 34°, or 36°, that it was a factor at 32°, and that it was not entirely absent at 34°. At 36° only traces of the disease appeared, and in common storage there was none. Experiments similar to those reported for three seasons in table 1 were also carried out in the seasons of 1925–26 and 1927–28, but no soggy break-down occurred. In

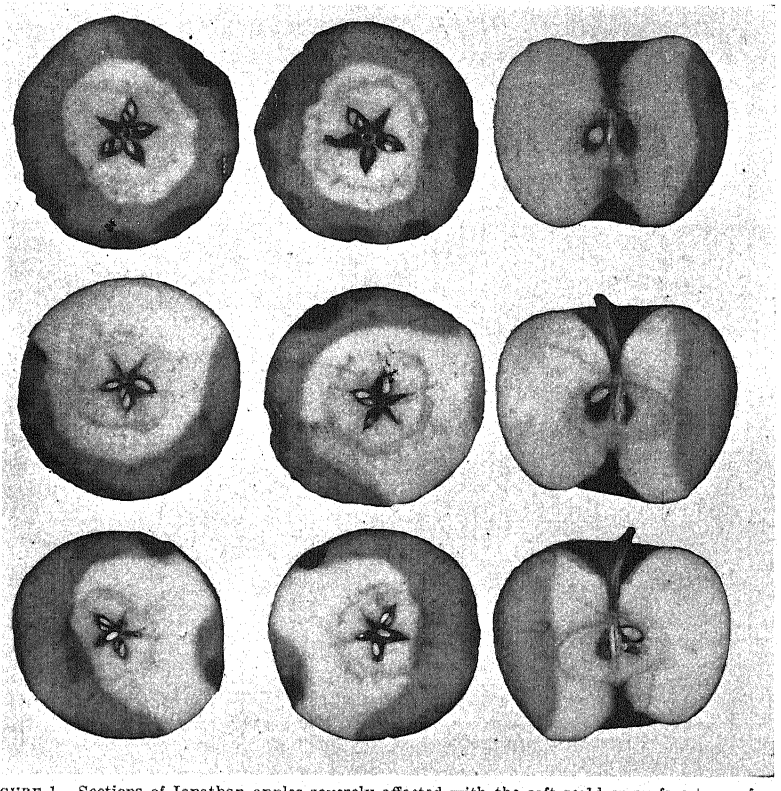


FIGURE 1.—Sections of Jonathan apples severely affected with the soft scald or surface type of soggy break-down. In most of the specimens shown the disease extends deeper into the cortex of the apple than is usual. (See fig. 8 for typical external appearance of soggy break-down in Jonathan.)

earlier work (*13*) the writers found extensive development of soggy break-down at 32° in each of three other seasons. The difference in the susceptibility of Jonathan in various years is thus very marked. The tendency to break-down in different lots of fruit given various delayed treatments also varied in different years. This factor is considered later.

The results of storing Jonathan at the two temperatures 30° and 36° F. for five additional storage seasons are shown in table 1. These studies also show complete control of the disease at 36°. Variation in the susceptibility of Jonathan in different years was again apparent, and dissimilar break-down tendencies between lots of fruit given different delayed treatments were also marked. The results as a whole show that at a temperature of 36° soggy break-down (fig. 1) was

completely eliminated in each of seven seasons and that it was kept under 1 percent in each of eight.

STUDIES WITH NORTHWESTERN GREENING

Storage experiments in which boxes of Extra Fancy Northwestern Greening apples were stored at various temperatures for each of two seasons are shown in table 2. In both seasons the fruit tested came from trees approximately 25 years old, and was picked at the peak of the picking season. The number of fruits per box ranged from 125 to 72, but the majority of the boxes contained between 113 and 88. No color distinction between green and yellow fruit was made. The study also includes 2 years of observations of this variety under common storage. Delay before storing took place in an open packing shed.

TABLE 2.—*Percentage of soggy break-down occurring in Extra Fancy Northwestern Greening apples* [1] *at different temperatures* [2]

Storage temperature (° F.)	Delay before storing	Slight break-down		Severe break-down	
		1924–25	1927–28	1924–25	1927–28
	Days	*Percent*	*Percent*	*Percent*	*Percent*
30	2	12.5	12.7	66.4	8.7
	7	12.5	16.8	17.3	8.4
	14	1.9	0	0	0
	21		13.4		41.3
32	2	12.2	28.4	41.4	43.2
	7	13.6	21.8	29.6	0
	14	0	2.6	0	.9
	21		14.9		4.4
34	2	1.5	42.7	1.0	17.3
	7	4.8	15.3	0	4.2
	14	0	0	0	0
	21		2.4		2.4
36	[3] 2	6.8	56.3	2.6	11.3
	7	1.6	5.1	.8	0
	14	0	1.7	0	0
	21		0		0
40	1	4.4		2.7	
	7	3.8		1.9	
	14	1.0		1.0	
29–60 [4] (common storage)	1	0		0	
	7	0		0	
	14	0		0	

[1] Picking date, October 13 in both seasons. Source of fruit: 1924, State orchard, western Iowa; 1927, Apple Grove orchards, central Iowa.
[2] Fruit examined February 18 for 1924–25 and March 3 for 1927–28.
[3] 1-day delay in 1924–25.
[4] Minimum and maximum temperatures for various months that fruit was in common storage: October, 44°–60° F.; November, 32°–54°; December, 32°–39°; January, 29°–34°.

The severity of the disease is expressed as slight break-down, which includes apples with an approximate total affected area not more than one-half inch in diameter; and severe break-down, which includes all specimens more severely affected. Severely affected apples frequently have 50 to 75 percent or more of their areas involved, and are always worthless. Slightly affected apples usually have some culinary value. At 36° F. Northwestern Greening shows a rather high proportion of slightly affected fruit, while at the lower temperatures the proportion of severely affected fruits is greater.

Severe break-down was usually most prevalent at the two lowest temperatures, 30° and 32° F. (fig. 2 and table 2). The results were similar for the fruit recorded under slight break-down in 1924, but for the 1927 fruit they were somewhat less consistent. At 40° the percentage of break-down was under 5 percent, and none occurred in common storage. The tendency for the fruit to decrease sharply in susceptibility with delayed storage is conspicuous in this variety; fruit given 2 and 3 weeks' delayed treatment was practically immune to soggy break-down.

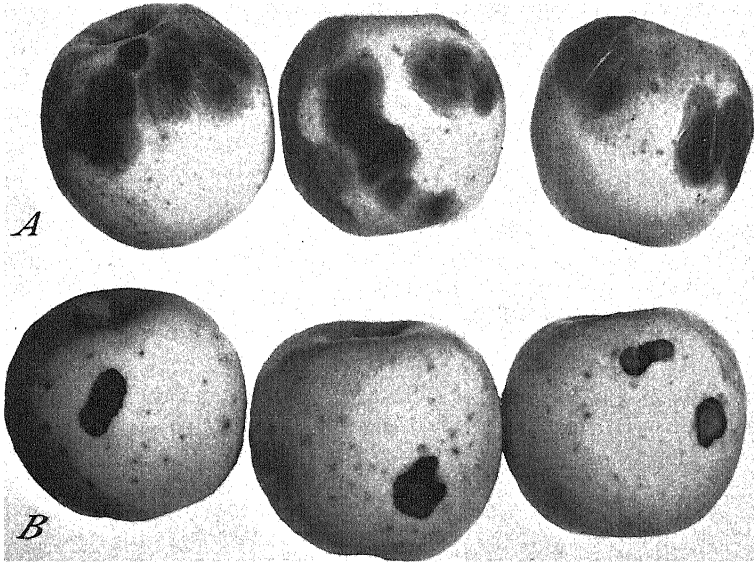


FIGURE 2.—Soggy break-down in Northwestern Greening: *A*, Severe soggy break-down occurring at 30° and 32° F.; *B*, slight soggy break-down in typical specimens stored at 36°. Like Jonathan, Northwestern Greening does not exhibit the internal type of soggy break-down.

Further evidence that storage temperature is a practical method of curtailing soggy break-down in Northwestern Greening is presented in table 3. The fruit used in this study was similar to that recorded in table 2 and received the same prestorage and storage treatment. Data for six seasons are presented on storage lots which were stored in most instances on the day of picking, or 1 or 2 days later. Except in one instance, break-down was most severe in fruit stored at 30° F. Fruit at 32° consistently yielded higher percentages than that at 34°, and the latter usually higher than fruit at 36°. No break-down occurred in common storage. The rather large percentage of break-down at 36° is indicative of the high degree of susceptibility of Northwestern Greening. This apple is regarded as the most susceptible variety thus far studied at Ames. The smaller amount of break-down noted in the 1925 data is probably the result of the delayed storage treatment of 11 days.

TABLE 3.—*Percentage of soggy break-down* [1] *occurring in Extra Fancy Northwestern Greening apples* [2] *during storage at different temperatures, 1924–29*

Year	Picking date	Delay before storing	Date examined	Break-down at storage temperature [3] of—			
				30° F.	32° F.	34° F.	36° F.
		Days		*Percent*	*Percent*	*Percent*	*Percent*
1924	Oct. 13	2	Feb. 18	66.4	41.4	1.0	2.6
1925	Oct. 9	11	Feb. 20	9.0	2.2	0	0
1926	Oct. 8	1	Jan. 13	41.6	27.3	11.2	5.6
1927	Oct. 13	2	Mar. 3	8.7	43.9	17.6	11.8
1928	Oct. 20	0	Mar. 22	46.3	28.8	10.4	8.7
1929	Oct. 8	0	Jan. 23	62.0	----------	----------	18.7

[1] Soggy break-down given as severe break-down; slight break-down excluded.
[2] The 1924 lot of fruit was from the State orchard in western Iowa; that for 1925–29 all came from Apple Grove orchards in central Iowa. In all cases fruit was from trees approximately 25 years old.
[3] No break-down occurred in common storage.

STUDIES WITH WEALTHY

In previous publications (*14*, *15*) the writers have reported the occurrence of soggy break-down in the Wealthy variety. A distinction was then made between soft scald and the internal type of soggy break-down; so that the data do not give a true picture of the severity of the latter disease in this variety. The internal type of soggy break-down was not recognized as such until some years later; and the earlier data (*14*, *15*) therefore include only the soft-scald type (fig. 3).

In the studies for the three seasons reported in table 4, the apples were picked in prime condition, were of good color, and of Extra Fancy grade. They were wrapped in oiled-paper wrappers and packed in boxes. The fruit in all cases was selected from old trees.

There was no soggy break-down of fruit in common storage, and when the disease occurred at 36° F., in most instances the percentage was low. Storage at 34° did not control the break-down satisfactorily, particularly when storage was delayed, and storage at 30° and 32° in most cases was even less favorable. In general the data indicate that 36° is the most desirable temperature for the prevention of soggy break-down, and that delayed storage up to a certain point increases the susceptibility of the fruit. Beyond this point, according to the 1928 data, delayed storage appears to increase resistance.

TABLE 4.—*Percentage of soggy break-down occurring in Wealthy apples during storage at different temperatures* [1]

Season	Picking date	Delay before storing	Break-down at storage temperatures [2] of—			
			30° F.	32° F.	34° F.	36° F.
		Days	*Percent*	*Percent*	*Percent*	*Percent*
1924–25	Sept. 17	1	----------	0.0	----------	----------
		7	----------	32.8	----------	----------
		14	----------	38.4	----------	----------
1926–27	Sept. 22	2	15.2	5.3	2.5	4.7
		9	22.0	12.3	6.0	1.2
1928–29	Sept. 5	2	1.7	2.3	3.0	0
		10	85.7	85.9	49.2	28.3
		14	79.6	89.8	44.4	11.3
		21	36.6	16.1	14.6	0

[1] Fruit examined in January in each season. Source of fruit: 1924 and 1926, northern Iowa; 1928, central Iowa.
[2] No break-down occurred in common storage.

STUDIES WITH WINTER BANANA

Attention was first called to the occurrence of soggy break-down in Winter Banana apples in 1933, when the writers observed a trace of the disease in one box of apples stored at 31° F. Results of storage tests for this variety are now available for three seasons (table 5). The apples came from northeastern Iowa; those tested in 1934 and 1935 were from trees 8 to 10 years old and those tested in 1936 were from trees approximately 25 years old, growing in a sandy loam soil. The fruit, of extra fancy grade, was packed and wrapped in oiled paper in 1934 and 1936, but was left unwrapped in 1935. The apples in the 1934 and 1936 seasons were picked on October 5 and in the 1935

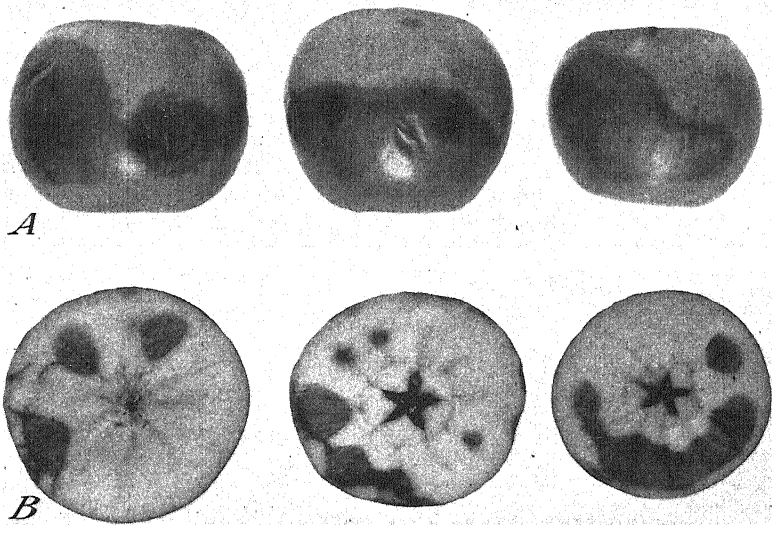


FIGURE 3.—Soggy break-down in Wealthy apples: *A*, Surface type resembling, in external appearance, the disease as it occurs on Jonathan and Northwestern Greening. Sections of such specimens frequently reveal also the internal type. *B*, Internal type in specimens exhibiting no external symptoms of the disease, though similar specimens frequently have both types of soggy break-down.

season on October 7. In 1935 and 1936 the fruit was considered normal in development; in 1934 it was slightly more mature than in the other two seasons. This might have been occasioned by extreme warm weather in the 1934 season.

It will be noted that the earliest stored fruit of the first season was most susceptible, and that there was a gradual decrease in the susceptibility of the apples given the 5 and 9 days' delay. With and following the 13-day delay there was usually marked decrease in susceptibility. The results for the second year differed from those of the first in that immediate storage and the shorter delays resulted in limited amounts of soggy break-down, while longer delays increased susceptibility. However, the tendency for the fruit given the longer delays (15 and 18 days) to increase in resistance, although not so marked, is apparent in the 1935 results, as it was in 1934. The data for storage at 36° are not complete, but the results indicate that Winter Banana apples are resistant to soggy break-down at this temperature.

TABLE 5.—*Percentage of soggy break-down occurring in Winter Banana apples during storage at different temperatures* [1]

Season	Apples stored at—		Delay at 50° F. before storing	Break-down in storage at 31° F.[2]	Season	Apples stored at—		Delay at 50° F. before storing	Break-down in storage at 31° F.[2]
	31° F.	36° F.				31° F.	36° F.		
	Number	*Number*	*Days*	*Percent*		*Number*	*Number*	*Days*	*Percent*
1934-35	113	125	1	73.5	1936-37	150	--------	0	1.4
	125	--------	5	60.2		163	--------	3	1.2
	290	--------	9	51.4		125	--------	6	7.2
	301	--------	13	16.6		163	--------	9	12.0
	301	--------	17	13.6		150	--------	12	5.4
	163	--------	21	4.3		138	--------	15	23.6
	150	--------	25	19.3		150	--------	18	29.5
1935-36	96	113	0	0		150	--------	21	55.0
	96	113	3	3.1		163	--------	24	16.6
	102	113	6	11.8		150	--------	27	3.3
	110	--------	9	68.2		163	--------	35	1.3
	109	--------	12	72.4		150	--------	42	0
	107	--------	15	57.8		150	--------	49	0
	102	--------	18	34.3					

[1] Fruit examined Feb. 15, 1935, Feb. 6, 1936, and Feb. 10, 1937.

[2] No break-down occurred in cold storage at 36° F., though only 1 test after 1 day delay was made in 1934-35 and 3 tests, after 0, 3, and 6 days' delay, respectively, were made in 1935-36. 1 box stored at 50° continuously in 1936-37 showed no break-down.

The results obtained in the third season confirm those of the second in that lowest break-down occurred with the shorter periods of delay, and susceptibility to break-down increased as the delayed storage period was increased to a certain degree. Highest susceptibility occurred with 21 days' delay in the 1936–37 season, and with 12 days' delay in 1935–36. The fruit of the first season was somewhat overmature and this probably accounts for the earlier stored samples of that year being more susceptible to soggy break-down.

Specimens of Winter Banana exhibiting the soft scald type of soggy break-down very frequently exhibit the internal type of break-down also, and both types occur concurrently in the same storage lots (*16*). These observations provide further evidence that the two types of soggy break-down are identical (fig. 4).

STUDIES WITH GOLDEN DELICIOUS

Golden Delicious is one of the most susceptible varieties and occasionally develops break-down at 34° and 36° F. Some results of experiments with this apple showing that a temperature of 36° controls soggy break-down have been published (*10*). It is sufficient to call attention to the fact that in these studies soggy break-down occurred severely at 30° and 32° F.; that 34° was unsatisfactory when storage was delayed 12 and 14 days; and that at 36° and in common storage the disease did not appear even though storage was delayed 3 weeks in some instances.

Further data for three seasons for fruits kept at a temperature of 31° F. likewise show the susceptibility of this variety to low-temperature break-down (table 7). Sufficient numbers of check boxes were kept at a temperature of 36° each year to confirm the resistance of Golden Delicious at the higher temperature.

RELATION OF DELAYED STORAGE TO SOGGY BREAK-DOWN

The effect of relatively short delays before storing and of prolonged periods of delay on the susceptibility of apples to soggy break-down varies with different varieties as well as with different seasons. In this discussion, then, consideration is given (1) to the effect of relatively

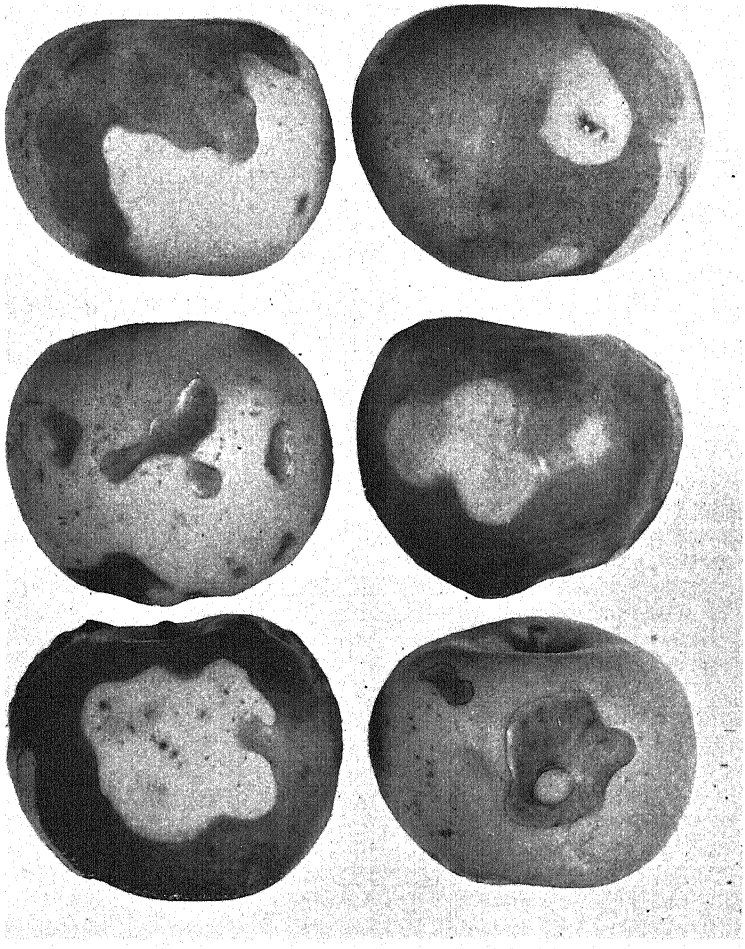


FIGURE 4.—Soggy break-down of Winter Banana apples. These specimens, although showing the typical surface type of soggy break-down, probably would also exhibit the internal type if sectioned.

short delays, and (2) to the effect of protracted delays on the subsequent development of soggy break-down in cold storage. The data presented are for typical seasons when the disease was prevalent in the varieties studied.

INFLUENCE OF RELATIVELY SHORT DELAYS

Unavoidable delays of a few days to several weeks sometimes occur in carrying out large-scale harvest operations. When this happens, the results may be expected to be considerably different from what

they would have been had the fruit been stored within 24 hours after picking.[4] Particularly is this true if the storage temperature is as low as 31° or 32° F.

From the results of the storage investigations at Ames, the writers have found it difficult to state definitely whether immediate storage or short delays result in greater susceptibility in Jonathan apples. In some instances immediate storage has made Jonathan more susceptible; in others delayed fruit has been more susceptible.

That delaying the storage of Jonathan may not necessarily increase susceptibility but, on the contrary, may decrease it, is indicated by the results shown in figure 5, *A*. Data are presented for four seasons, and these may be considered to give about the average picture over a period of years. In two seasons short delays of approximately a week to 10 days increased the tendency to break-down; in two other seasons a delay of 1 week as compared to immediate treatment, decreased susceptibility. With one exception, 2 to 3 weeks' delay made Jonathan more resistant, or about as resistant as immediate treatment. These observations show that susceptibility to low-temperature breakdown in Jonathan may be either positively or negatively associated with short delays before storage, according to the particular season in which the fruit is grown. Further evidence supporting this statement is given in tables 1 and 2.

That delayed treatment may definitely make apples more resistant to soggy break-down is indicated by results depicted in figure 5, *B*. The results for Northwestern Greening are typical for this variety (table 3). Results for the one season with Winter Banana, though similar to those for Northwestern Greening, are the reverse of those obtained in 2 other years (table 5); so that no definite statement as to the performance of this variety can yet be given. It may, like Jonathan, give on result one season and the opposite result another.

The usual or expected results of delaying storage of Golden Delicious and Grimes Golden apples are shown in figure 5, *C*. For these two varieties immediate storage, or a very short delay of 2 to 3 days, usually gives better results than longer delay. The two seasons' results for these varieties exemplify the general rule for the occurrence of soggy break-down. However, exceptions to this rule for Grimes Golden and possibly Golden Delicious may be expected. For example, data for 1924 (*15*) showed that Grimes Golden given only 24 hours' delay became susceptible to break-down, and data for another season showed that when grown during the very warm summer of 1934 under heavy irrigation it became very susceptible under immediate treatment (table 6). Regardless of exceptions, however, it is reasonably certain that delays of 1 to 3 weeks in the storing of Grimes Golden and Golden Delicious will result in higher susceptibility to soggy break-down than will immediate storage.

INFLUENCE OF PROLONGED DELAYS

As in the case of short delays, the effect of prolonging the period of delayed storage on the subsequent behavior of apples in storage varies considerably between varieties and seasons. In studying this problem, testing and observation over a period of years with susceptible varieties was necessary.

[4] The writers have data which indicate that a difference of 24 hours in the storing of Jonathan may result in marked differences in susceptibility.

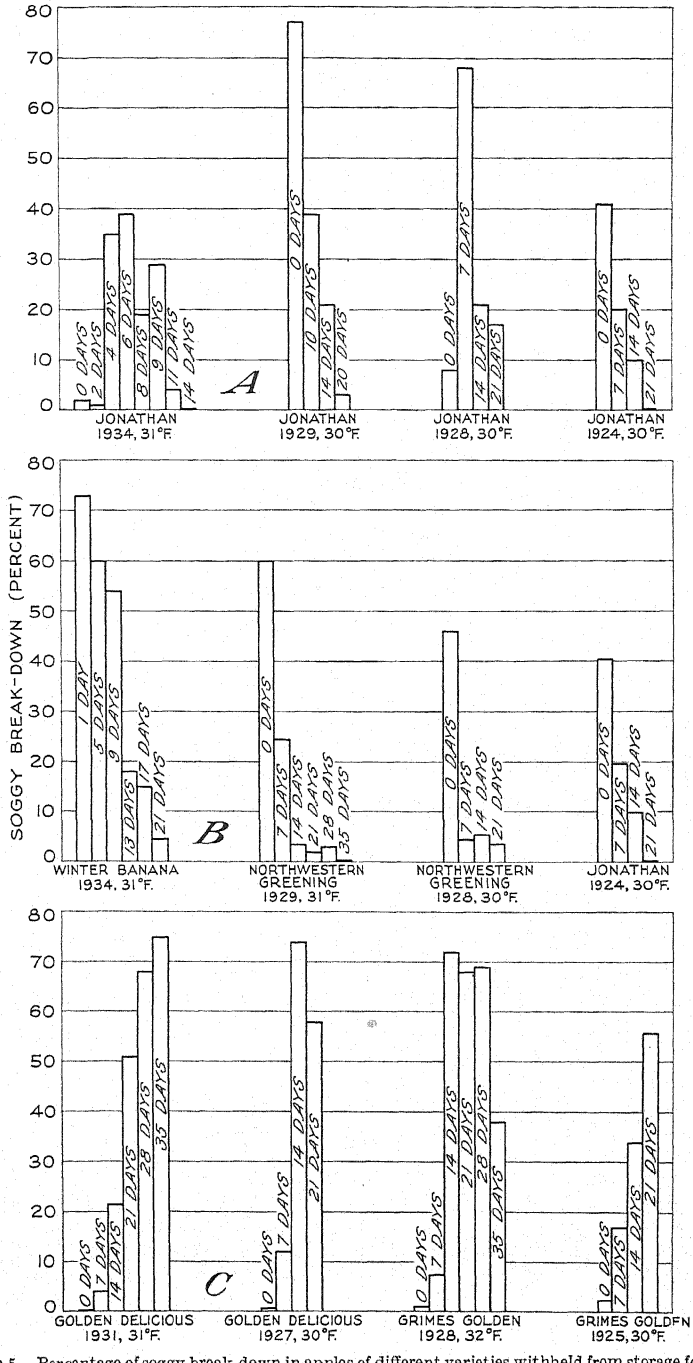


FIGURE 5.—Percentage of soggy break-down in apples of different varieties withheld from storage for various lengths of time and then stored at the temperatures shown. *A*, fruit held at 50° F. before storage, except in 1924 when it was held at outdoor temperatures; *B*, fruit held at 50° in 1929 and 1934 and at outdoor temperatures in 1924 and 1928; *C*, fruit held at 50° in 1928 and 1931 and at temperatures prevailing in an open packing house in 1925 and 1927.

Experimental studies since 1924 have shown that keeping Jonathan at ordinary outside temperatures 3 to 4 weeks or longer before cold storing makes it practically immune to soggy break-down.[5]

Results with Grimes Golden

The effect of protracted periods of delay on the development of soggy break-down in Grimes Golden for each of 5 years is recorded in table 6. After 6 weeks of delayed treatment in 1928 in each of three lots stored at different temperatures, there may be noted a definite breaking point in the susceptibility of the stored fruit. Six weeks' delay resulted in similar reductions in the susceptibility of the fruit in 1931 and 1934, while 5 and 7 weeks' delay yielded similar results in 1929 and 1930. In nearly all instances, therefore, protracted delays reduced the tendency to soggy break-down after definite periods of time. Periods of delay greater than 5 to 7 weeks did not increase susceptibility to this type of break-down.

TABLE 6.—*Effect of length of period of delay before storage upon the percentage of soggy break-down occurring in Grimes Golden apples* [1] *during storage at different temperatures in five seasons*

Lot No.	Delay at 50° F before storing	Break-down in season and at temperature indicated								
		1928–29			1929–30		1930–31		1931–32	1934–35
		30° F.	32° F	36° F	30° F	36° F	31° F	36° F	31° F.	31° F.
	Weeks	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
1	0	3.8	0.4	0.4	4.7	0.0	0 0	0.0	0 0	68.5
2	1	.8	6.5	.6	------	-- ---	1.5	0	.6	97.0
3	[2] 2	67.8	73.5	4.0	24.6	0	5.6	0	1.7	63.4
4	3	60.3	67.9	8.7	41.0	0	9.3	0	2 4	43.7
5	4	63.4	69.3	21 4	7.4	0	13.4	0	2.2	44.8
6	5	68.5	38.0	8.1	2.3	0	5.6	0	2.5	36.1
7	6	.7	3.5	0	0	0	16.9	0	1.2	7.5
8	7	.8	0	0	0	0	2.2	0	0	6.1
9	8	.6	0	0	0	0	1.5	0	0	6
10	9	0	0	0	--------	--------	--- --	---- --	0	0
11	10	0	0	0	--------	--------	-----	- ----	- - -	0
12	11	0	0	0	--------	--------	--- -	--	- --	0
13	12	0	0	0	--------	--------	--	--- ----	- -	- --

[1] Source of fruit: 1928 and 1929, old trees, Apple Grove orchards, central Iowa; 1930 and 1931, young trees, Highland fruit farm, western Iowa; 1934, young trees, Schurk orchard, southeastern Iowa. Growing conditions: 1928 and 1929, temperature moderate, rainfall abundant; 1930 and 1931, temperature excessively high, soil moisture unusually low; 1934, temperature very high, orchard under irrigation. Picking dates: Sept. 25, 1928; Sept. 26, 1929; Sept. 16, 1930; Sept. 22, 1931; Sept. 25, 1934. Fruit examined: Jan. 24, 1928; Jan. 29, 1929; Apr. 1, 1930; Feb. 19, 1931; Feb. 15, 1934. 1 box in each test.

[2] 10 days' delay in 1929–30.

In table 6 it may be noted that in 1934 the marked predisposition to soggy break-down in promptly stored Grimes Golden was accompanied by low break-down tendencies under prolonged delayed treatments. This fruit from a heavily irrigated orchard in southern Iowa was grown during the warmest summer on record and was picked when very mature, yet it improved in resistance with the longer delayed treatments. It appears, therefore, that Grimes Golden becomes markedly less susceptible to soggy break-down when given a 6-week

[5] It seems advisable to point out that the practice of delaying the storage of fruit either for short periods or for long periods is not recommended in any instance as a measure for the prevention of soggy break-down. The experiments on delayed storage have been conducted mainly for the purpose of acquiring information pertaining to the nature of this disease. Delaying storage of apples may make them more resistant to soggy break-down, but it may also make them more susceptible to other diseases, such as apple scald, Jonathan spot, bitter pit, mealy break-down, or to loss in flavor, mealiness, etc.

prestorage treatment at 50° F., and practically immune with still longer, similar prestorage treatments.

Harding's respiration studies (*5*) are of particular significance in view of the foregoing results. He found that high respiratory activity (in the 1928–29 and 1929–30 fruit, table 6) was correlated with high susceptibility to soggy break-down. In other words, both the respiration rate and the liability to soggy break-down were noticeably lowered after approximately 6 weeks of storage at 50° F.

Results with Golden Delicious

In experiments with Golden Delicious, comparable to those carried on with Grimes Golden and Jonathan, the results were similar but less conclusive. Data for 3 years of observations are reported in table 7. Relatively long delays of 8 to 12 weeks at 50° F. somewhat decreased the severity of soggy break-down in the 1931 and 1934 fruit. After 9 and 10 weeks of delayed treatment in 1931 there was a distinct lowering of susceptibility, the percentages being 31.5 and 44.6 respectively, as compared to 57.3 for the 8 weeks' treatment. There was a gradual decrease in susceptibility beginning with the 7 weeks' treatment, but the differences were less marked than with Grimes Golden. Decreases in susceptibility to severe break-down in the 1934 fruit are similar to those in the 1931 fruit. As the tendency to severe break-down decreased in the 1934–35 fruit, the tendency to slight break-down increased.

Table 7.—*Effect of length of period of delay before storage upon the percentage of soggy break-down occurring in Golden Delicious apples* [1] *during storage at 31° F. in 3 seasons between 1930 and 1935*

Lot No.	Delay at 50° F. before storing	Break-down in—						
		1930–31			1931–32	1934–35		
		Slight	Severe	Total	Total	Slight	Severe	Total
	Weeks	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
1	[2] 0	0.0	0.0	0.0	0.0	7.2	21.7	29.0
2	1	0	0	0	3.3	5.6	63.0	68.6
3	2	0	0	0	22.1	16.2	48.5	64.7
4	3	2.5	0	2.5	51.0	7.4	77.8	85.3
5	4	7.3	15.3	22.6	67.7	6.6	71.5	78.2
6	5	12.4	32.8	45.0	75.5	4.2	90.9	95.2
7	6	10.4	13.9	24.4	79.6	7.4	84.6	92.0
8	7	13.5	24.5	38.1	61.3	10.9	86.2	97.0
9	8	9.0	37.7	46.7	57.3	6.4	83.4	89.7
10	9	----------	----------	----------	31.5	14.4	72.5	86.9
11	10	----------	----------	----------	44.6	13.1	80.9	94.0
12	11	----------	----------	----------	----------	46.7	33.3	80.0
13	12	----------	----------	----------	----------	35.6	44.2	79.8

[1] Source of fruit: 1930, Apple Grove orchards, central Iowa; 1931, Highland fruit farm, western Iowa; 1934, Schurk orchards, southeastern Iowa. Growing conditions: 1930 and 1931, temperature high, soil moisture low; 1934, temperature very high, orchard heavily irrigated. Picking dates: Oct. 6, 1930; Oct. 7, 1931; Sept. 18, 1934. Fruit examined: Mar. 30, 1930; Feb. 25, 1931; Feb. 11, 1934. Number of boxes: 1931 and 1934, 1; 1932, 2.

[2] Stored same day picked in 1930 and 1931; 6 days' delay in 1934.

The difference between Grimes Golden and Golden Delicious in their response to long-delayed treatment must be considered in relation to varietal characteristics, i. e., date of maturity and length of storage season. Grimes Golden, for example, matures relatively early and begins its storage period early, whereas Golden Delicious matures late, at a

time when the average growing temperatures are lower. The conditions to which the two varieties are subject during the maturation period are decidedly different, and the fact that their ripening periods are not coincident may alone be sufficient to account for different responses in storage. Experiments similar to the foregoing were carried out with Golden Delicious in three other seasons, but no soggy break-down developed, indicating again that seasonable differences in the susceptibility of apples must be considered in studying this disease.

Results with Northwestern Greening

Results with Northwestern Greening for the season 1929–30 are given in table 8. The fruit, which was obtained from central Iowa, was selected and handled as in former seasons. The data indicate a marked lowering in the susceptibility of the apples when storage was delayed 2 weeks at 50° F. With longer delay practically no break-down developed at either 31° or 36° F. The results are similar to those for Jonathan, which consistently exhibits resistance with delayed treatments longer than 3 and 4 weeks.

RELATION OF MATURITY OF FRUIT ON DATE OF PICKING TO SUSCEPTIBILITY TO SOGGY BREAK-DOWN

Earlier results published by the writers (*13*) indicated that the maturity of Jonathan on the date of picking influences its susceptibility to soggy break-down. An interpretation of the earlier results, together with those for three other seasons, is summarized in table 9. The pickings recorded for each year extended throughout the commercial picking season for Jonathan, so that intermediate dates represent fairly closely the optimum picking dates. The term "highest maturity" indicates the degree of maturity attained on the latest picking date; the terms "intermediate" and "lowest maturity" refer to maturity attained on intermediate and earliest picking dates. All samples were stored at 32° F.

TABLE 8.—*Effect of length of period of delay before storage upon the percentage of soggy break-down* [1] *occurring in Northwestern Greening apples during storage at 31° and 36° F., 1929–30*

Lot No.	Delay at 50° F. before storing	Apples [2]	Break-down at storage temperature of—		Lot No.	Delay at 50° F. before storing	Apples [2]	Break-down at storage temperature of—	
			31° F.	36° F.				31° F.	36° F.
	Weeks	*Number*	*Percent*	*Percent*		*Weeks*	*Number*	*Percent*	*Percent*
1	(4) 0	100	62.0	18.7	6	5	96	0.0	0.0
2	1	72	23.6	1.4	7	6	64	0	0
3	2	138	3.7	0	8	7	100	0	0
4	3	96	1.0	0	9	8	88	0	0
5	4	96	3.1	0					

[1] Fruit examined January 23.
[2] Number also indicates size of fruit.
[3] Stored same day picked, October 8.

In Jonathan, the highest degree of maturity as indicated by late picking appears not to be correlated with greatest susceptibility; and fruit picked at the beginning of the commercial season, or before it had become overmature, appears to be more susceptible to soggy break-down. These results agree in part with those reported by Tiller and

Chittenden (*20*) of New Zealand, who found that overmature Jonathan apples were less susceptible to "deep scald" than fruit of lesser degrees of maturity.

TABLE 9.—*Relation of degree of maturity of the fruit to soggy break-down susceptibility in Jonathan apples stored at 32° F.*

Season	Pickings per season	Degree of maturity associated with lowest susceptibility	Degree of maturity associated with highest susceptibility
	Number		
1919–20	5	Highest	Lowest.
1920–21	6	Lowest	Intermediate.
1922–23	5	Highest	Lowest.
1923–24	4	Lowest	Intermediate.
1924–25	3	Highest	Do.
1926–27	4	Intermediate and highest	Lowest.

The results of varying the picking maturity of Jonathan throughout three seasons are given in table 10. In 1923, the highest resistance was found in the earliest samples harvested on September 18; the lowest resistance in fruit of intermediate maturity harvested on September 24; and progressive increases in resistance were noted in the two succeeding pickings of fruit.

The 1924 results were similar in that highest maturity resulted in low susceptibility. Because of the slight occurrence of soggy break-down the data for 1926 give less information, but they do indicate greatest susceptibility in one of the most immature samples.

The value of these results with Jonathan lies mainly in that they show that picking maturity seems to affect susceptibility to soggy break-down, and that storage behavior may be directly related to the maturity attained on the tree.

TABLE 10.—*Percentage of soggy break-down occurring in apples of several varieties harvested at various stages of maturity and stored at 32° F.*

Variety	Year	Picking date	Break-down after indicated delay before storing of—			
			None	1 week	2 weeks	3 weeks
			Percent	*Percent*	*Percent*	*Percent*
Jonathan	1923	Sept. 18	0.0	4.5	----------	----------
		Sept. 24	45.5	82.2	----------	----------
		Sept. 29	34.6	14.0	----------	----------
		Oct. 4	13.5	12.3	----------	----------
	1924	Sept. 30	10.2	8.6	3.0	0.0
		Oct. 7	18.3	3.4	.3	0
		Oct. 14	4.8	2.9	1.7	0
	1926	Sept 20	0	3.4	0	0
		Oct. 2	0	0	0	0
		Oct. 5	0	0	0	0
		Oct. 12	0	0	0	0
Wealthy	1923	Aug. 27	0	8.9	12.0	----------
		Sept. 4	0	4.0	1.0	----------
		Sept. 11	0	2.0	0	----------
	1924	Aug. 28	0	0	.6	----------
		Sept. 3	0	.6	4.3	----------
		Sept. 9	0	0	10.4	----------
		Sept. 17	0	22.8	31.8	----------
Grimes Golden	1928	Sept. 7	0	----------	----------	44.9
		Sept. 11	0	----------	----------	65.3
		Sept. 14	0	----------	----------	61.4
		Sept. 20	0	----------	----------	89.0
		Sept. 25	.4	----------	----------	67.9
Northwestern Greening	1923	Sept. 12	0	4.4	4.7	----------
		Sept. 19	8.0	3.6	----------	----------
		Sept. 25	19.3	28.8	2.0	----------
		Oct. 17	51.2	----------	----------	----------

With Wealthy, the results for the two seasons are conflicting. In 1923 the most immature fruit was the most susceptible, and in 1924 the most mature fruit was the most susceptible.

With Grimes Golden, the highest susceptibility was found in fruit picked on September 20. As judged by the usual methods for determining maturity, this date was considered the optimum picking time of the season. It should also be noted that very immature fruits of Grimes Golden, picked as early as September 7, were highly susceptible

With Northwestern Greening, undermaturity was associated with a much higher degree of resistance than overmaturity.

EFFECT OF CONTINUOUS MOVEMENT OF STORAGE-ROOM ATMOSPHERE AND OF STORING IN OPEN CONTAINERS ON SOGGY BREAK-DOWN

In 1924 the writers reported studies (*13*) in which recirculation of the storage-room atmosphere gave good control of soggy break-down in one season and only partial control in another. The apples used were Extra Fancy Jonathan, wrapped and packed in standard boxes. Other researches (*1, 8*) pertaining to the application of ventilation as a control measure in Jonathan, showed that circulating the storage-room atmosphere and using ventilated containers did not give consistent results. The results of further studies on the effect of aerating storage rooms and the use of open containers are given in table 11. Each lot of fruit consisted of a quantity equal to a standard apple box. The fruit was of Extra Fancy grade and was medium in size. The type of open basket used is shown in figure 6.

With one exception all of the eight boxes under air movement developed more soggy break-down than the boxes in the unaerated room. A comparison of the efficiency of the open-wire-basket method of storing under moving-air conditions with the conventional apple-box method in a room with still air showed in six of eight instances more break-down in the wire-basket containers. When open and standard containers both under aeration were compared, conflicting results were obtained.

TABLE 11.—*Effect of aerating storage rooms upon the percentage of soggy break-down occurring in Jonathan and Grimes Golden apples stored at 30° and 32° F. in two types of containers, season 1923–24*

Variety and storage temperature (°F.)	Picking date	Delay before storing	Break-down in storage room, air artificially circulated		Break-down in storage room, air not circulated, apples wrapped; in standard boxes
			Apples not wrapped; in open-mesh wire baskets	Apples wrapped; in standard boxes	
		Days	*Percent*	*Percent*	*Percent*
Jonathan, 32	Sept. 18	2	0.0	6.4	0.0
		8	52.6	69.8	63.2
	Sept. 24	2	14.0	14.9	4.1
		8	58.7	48.5	35.0
	Sept. 29	4	24.0	31.3	8.4
		7	9.6	2.3	3.0
	Oct. 4	4	23.7	16.5	.9
		8	35.3	26.0	18.8
Grimes Golden:					
30	Sept. 25	14	16.1	67.8	----------
		21	13.6	60.3	----------
32	---do----	14	12.2	73.5	----------
		21	2.5	67.9	----------

Strikingly different results were obtained with Grimes Golden at 30° and 32° F. In all instances the wrapped fruits in standard boxes developed more soggy break-down than those in open-mesh baskets. It should be noted, however, that the Grimes Golden in the open



FIGURE 6.—Method of storing Jonathan apples in wire baskets in aeration studies. Fruit in these baskets, under continuous atmospheric movement and at the same temperature, became more severely affected with soggy break-down than wrapped fruit in standard boxes in rooms without atmospheric movement. Dissimilar results were obtained with Grimes Golden.

baskets were somewhat shriveled at the end of the experiment, although not sufficiently so to affect their market value. The Jonathan fruits were not noticeably shriveled. The results tend to confirm those reported by the writers in an earlier experiment (*15*), in which storing Grimes Golden in open-wire baskets gave complete control of soggy break-down.

The comparison of fruits in open and closed containers with and without circulating storage-room atmosphere is not comprehensive enough for final conclusions. It may reasonably be expected, however, that the methods of storing and aerating used will not control soggy break-down in Jonathan. The results with Grimes Golden give more promise, although complete control was not obtained.

OTHER ETIOLOGIC FACTORS

Throughout the course of the investigations reported above, certain other etiologic factors of soggy break-down have received consideration. These include the effect of (1) fruit color, (2) nitrate fertilizers, (3) climatic conditions, and (4) size of fruit. Studies of all of these factors are not complete and final conclusions cannot be given.

TABLE 12.—*Effect of degree of fruit color upon the percentage of soggy break-down occurring in Jonathan and Northwestern Greening apples* [1] *during storage at different temperatures*

Variety	Picking date	Storage temperature	Color designation [2]	Stored immediately		Storage deferred 2 weeks	
				Apples	Break-down	Apples	Break-down
		° F.		*Number*	*Percent*	*Number*	*Percent*
Jonathan	Oct. 2, 1928	30	High	134	8.2	138	21.7
			Low	138	0	150	10.7
		32	High	110	1.8	113	16.8
			Low	138	0	149	8.1
		34	High	150	0	138	5.1
			Low	113	0	110	3.6
Northwestern Greening	Sept. 24, 1925	30	Yellow	217	54.3	----	----
			Green	288	16.0	----	----
		32	Yellow	151	84.1	----	----
			Green	255	30.9	----	----
		34	Yellow	173	74.5	----	----
			Green	250	36.4	----	----

[1] Date of examination, March 1 each year. Apples in oiled wraps in single boxes.
[2] The term "high color" for Jonathan indicates fruit completely colored or with 100-percent color; "low color," fruit 25 to 50 percent colored. In the case of Northwestern Greening, the yellow fruit was a deep golden yellow, the green about an apple-leaf green. Both yellow and green fruit were selected from the same trees on the same day.

Some effects of the degree of color of the fruit on susceptibility are given for Jonathan and Northwestern Greening in table 12. The results for one season suggest that color may influence susceptibility to some extent. Jonathan with 100-percent color in storage at 30° F. was less resistant than fruit with only 25- to 50-percent color. In Northwestern Greening at all three temperatures, fruit of full yellow color was much more susceptible than leaf-green fruit. However, the green fruit was not entirely resistant. In other experiments with Grimes Golden and Golden Delicious the writers have noted that green fruit was often more resistant than well-colored fruit, but not invariably so.

The results of 3 years' work on the influence of nitrogenous fertilizers on soggy break-down have been published (*12*), and while further studies have been made, the results merely confirm those already reported. In the earlier work (*12*) it was found that in wet years nitrate fertilizer seemed to increase susceptibility considerably in both Grimes Golden and Jonathan, but not in the 2 subsequent dry years (1930 and 1931). Similar experiments during 1932 and 1933

in four different orchards, with Grimes Golden, Jonathan, and Golden Delicious, have shown no further consistent increase in susceptibility of these varieties when the trees were fertilized with nitrate of soda. However, the continuous abnormal growing conditions from 1930 to 1933 resulting from high temperatures and drought, should be considered in interpreting the results, for under such conditions apples become more resistant. Drought conditions made it necessary to discontinue the study in 1934. In view of these facts, the writers do not consider the question of the relationship of nitrogenous fertilizers to the liability of apples to soggy break-down adequately answered.

In most of the varieties that are susceptible to soggy break-down it has been observed that the size of the fruit is of little consequence. In the majority of the tests the apples were sorted according to size before they were packed, so that the size factor has been definitely under observation throughout. The results to date indicate that small apples are fully as liable to soggy break-down as large ones. The one exception thus far noted is Northwestern Greening. The results of experiments with two size classes of this variety are recorded in table 13. The size class 64 to 104 proved to be much more susceptible than class 113 to 150. The differences noted between large and small fruit can probably be explained on the basis of maturity, since large Northwestern Greenings are generally considered to be more advanced in maturity on the tree than small Greenings. The range in size in this variety averages wider than most varieties. Large Northwestern Greenings, then, appear to be more susceptible to soggy break-down than small.

TABLE 13.—*Effect of fruit size upon the percentage of soggy break-down occurring in Northwestern Greening apples* [1] *during storage at different temperatures, season 1928–29*

Lot No.	Delay at 50° F. before storing	Break-down in large fruit (sizes 64 to 104) [2]				Break-down in small fruit (sizes 113 to 150) [2]			
		30° F.	32° F.	34° F.	36° F.	30° F.	32° F.	34° F.	36° F.
	Days	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
1	0	62.8	57.5	48.5	6.3	46.3	28.8	10.4	8.7
2	7	15.9	12.8	12.5	2.0	5.1	8.9	0	0
3	14	21.6	1.0	5.8	1.9	4.9	2.4	.8	0
4	21	3.8	.7	2.9	5.3	2.4	2.4	0	.8

[1] Date picked, Oct. 20; date of examination, Mar. 22.
[2] Various treatments resulted in no break-down in common storage.

THE PERIOD OF DEVELOPMENT OF SOGGY BREAK-DOWN

The development period of soggy break-down in Grimes Golden was reported in an earlier publication (*15*) to extend approximately from December 12 to February 15. The disease developed rapidly between January 11 and February 15, but there was no subsequent development up to May 12.

The method of storing Jonathans in open wire baskets, described above, afforded an opportunity to note the periodic development of soggy break-down in this variety. The fruit in the open wire baskets used in the aeration experiment (table 11) was carefully inspected at weekly intervals in the storage room to determine the initial appearance and the severity of the disease in all lots under treatment. The

data obtained (table 14) show that the dates of first appearance of the disease in the different baskets ranged from November 20 to January 3. It is evident, therefore, that soggy break-down may appear as early as 2 months after storage. Early appearance of the disease and high susceptibility seem to be associated.

TABLE 14.—*Date of initial appearance and maximum percentage of soggy break-down attained in Jonathan apples in open wire containers when stored immediately and after 1 week's delay, at 32° F., season 1923–24*

Lot number	Picking date	Break-down under immediate storage		Break-down when storage was delayed 1 week	
		Initial appearance	Maximum	Initial appearance	Maximum
			Percent		*Percent*
1	Sept. 18	None	0	Nov. 20	52.6
2	Sept. 24	Dec. 3	14.0	do	58.7
3	Sept. 29	do	24.0	Jan. 3	9.6
4	Oct. 4	Dec. 18	23.7	Dec. 3	35.3

Other studies with Jonathan have indicated that the period of development terminates toward the end of the commercial storage period. For example, published data (*13*) show that very little break-down developed between February 17 and April 1 in 1920, or between January 28 and March 28 in 1921. Other observations (table 15) show no development after January 26 in 1924, and still others show practically none after February 1. The period of development of the disease in Jonathan terminates rather abruptly, as it does in Grimes Golden. Although less is known of the period of development in other varieties, there are indications that it is similar to that of Grimes and Jonathan.

Further evidence that soggy break-down has a definite period of evolution in Jonathan was obtained by observing the increase in area of diseased tissue in marked specimens. On November 15, when break-down was first noted, 100 Jonathan apples were removed from storage, the affected portions were outlined with a sharp-pointed, moistened indelible pencil and the fruit was then returned to storage. The method of outlining the diseased areas and the progressive development from November 15 to December 3 are shown on two typical specimens in figure 7. In no case did the diseased areas expand after January 1.

TABLE 15.—*Extent of soggy break-down in Jonathan apples, stored at 32° F. after different periods of delay; examined on Jan. 26 and Mar. 12, 1924*

Lot No.	Picking date	Apples	Delay before storing	Break-down on—		Lot No.	Picking date	Apples	Delay before storing	Break-down on—	
				Jan. 26	Mar. 12					Jan. 26	Mar. 12
		Number	*Days*	*Percent*	*Percent*			*Number*	*Days*	*Percent*	*Percent*
1	Sept. 30	688	1	10.2	0	7	Oct. 7	676	14	.3	0
2		652	7	8.6	0	8		675	21	0	0
3		663	14	3.0	0	9	Oct. 11	688	1	4.8	0
4		651	21	0	0	10		626	7	2.9	0
5	Oct. 7	639	1	18.3	0	11		651	14	1.7	0
6		651	7	3.4	0	12		676	21	0	0

On January 14 of the same season 500 diseased Jonathan apples were marked and immediately returned to cold storage. An examination on February 15 revealed no further increase in the marked areas. It is recognized that the conditions to which the marked apples were subjected were not the same as those of other fruit kept continuously in storage containers. However, the results indicate that the advance of soggy break-down may be easily checked and that this disease has a rather definite period of development. In the latter respect it differs from apple scald, Jonathan spot, and mealy break-down, which continue to develop before and after the fruit is removed from storage. This information is of interest to commercial

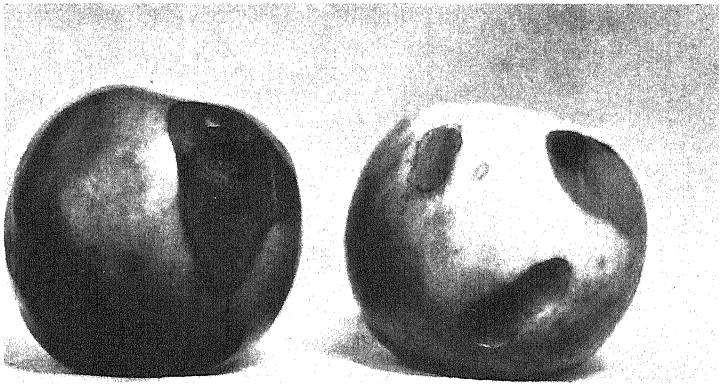


FIGURE 7.—Jonathan apples marked to show development of soggy break-down. Affected areas did not increase in size from December 3 to April 3. Apples were put back into cold storage immediately after marking. Typical external appearance of soggy break-down of Jonathan. (See fig. 1 for internal appearance of soggy break-down.)

storage operators and growers who may naturally suppose that most storage diseases of apples increase progressively in severity as the storage period is prolonged.

DISCUSSION

The observations recorded on the effects of picking maturity and delayed storage suggest that soggy break-down in apples may be associated with the stage of metabolism attained by the fruit at the time it is stored. Further evidence of this is brought out in the respiration studies of Harding (*4*, *5*) on large samples of Grimes Golden, and the storage studies carried out concurrently on similar fruit by Plagge (*11*). Harding found that respiratory activity in Grimes Golden accelerated rapidly at 50° F. during the first few weeks after picking. If actively respiring fruits of Grimes Golden were stored at 30°F., they subsequently became highly susceptible to soggy break-down; but if the fruits were stored before a high respiratory level was attained, or after it had subsided, they were resistant to the disease. When the fruit was stored at 36°F., regardless of its respiratory activity, it was resistant.

Other evidence that the stage of metabolic activity is important is shown by the results of many storage experiments with Jonathan and

Grimes Golden. In earlier experiments (*13*) with Jonathan, the writers observed that apples which had become severely affected with Jonathan spot were only slightly susceptible to soggy break-down. More recent studies confirm these observations. Jonathan fruits delayed 2 to 3 weeks before storing develop much spot and little or no soggy break-down, while those stored more promptly develop little Jonathan spot but more break-down. Jonathan apples susceptible to spot have already attained a high metabolic rate when stored, since this disease is closely allied to senility. Fruit that is susceptible to soggy break-down, therefore, is probably not so far advanced in its respiratory cycle when stored as that which is susceptible to Jonathan spot.

Similarly, a study of the tendency of Grimes Golden to apple scald and soggy break-down yields evidence that the stage of metabolism on the date of storage is indicative of its susceptibility to soggy break-down. In studies (*4*, *11*) in which respiratory activity in Grimes Golden was shown to be closely correlated with break-down susceptibility, break-down fruit was not affected with apple scald, or vice versa. This was observed a number of times with Grimes Golden and other varieties, and recently Brooks and Harley (*2*) have called attention to a similar result with Grimes Golden.

The results with Winter Banana further indicate that delayed storage may give different results in different seasons, and that no rule can be formulated as to the extent of delayed treatment that will result in maximum susceptibility.

The results of the storage-temperature studies over a period of years emphasize the need for raising slightly the apple storage temperatures used in commerce. Experiments year after year have shown that susceptible varieties store satisfactorily at 36° F., and that similar fruit may be severely affected with soggy break-down when the temperature is lower. Some may prefer to emphasize prompt storage rather than change the temperature, but such a practice is not always possible, and it will not serve as a satisfactory remedy in all cases, or even consistently in a few. The alternative is to store apples at 35° to 36° F. instead of at the conventional 31° to 32° range. Other reasons for making this change in the storage temperature were presented in an earlier publication (*17*).

Results of aeration studies with Jonathan, in which the storage-room atmosphere was kept continuously in motion around wrapped fruit in closed boxes, as well as around unwrapped fruit in mesh-wire baskets, offer little hope that circulating the storage-room atmosphere would be of value in the control of soggy break-down. Although some beneficial results from aeration methods have been recorded, it is believed that these may have followed from the more rapid rates of cooling in open containers and aerated storage rooms, with the net result of a changed metabolism, or from the resulting more even temperature control rather than from the removal of products of respiration. Whitehouse (*21*) pointed out that 160 hours (6½ days) was required to cool the center of a box of wrapped apples to 34° from 70° when the room temperature was 32° F., and that unwrapped fruit in boxes cooled more rapidly than wrapped. Moreover, a difference in time of only 3 days in the rate of cooling may markedly change the break-down susceptibility, especially when apples are in a high metabolic state.

The removal of certain harmful products of respiration deserves some consideration, particularly in the light of recent studies by Kidd and West (7), who point out that a toxic substance (probably ethylene) from ripe applies accelerates respiratory activity. But in the experiments described above Jonathan fruits sometimes developed more break-down in open containers than in closed boxes, and more in closed boxes under moving air than in still air. In these experiments, recirculating the storage-room air would probably not remove from the apples small traces of certain gases such as ethylene.

SUMMARY

Experiments carried on over a period of years on the effects of various causal factors of soggy break-down in apples, including picking maturity, storage temperature, short and long prestorage delays, and aeration are reported.

The maturity of the fruit on the picking date was shown to affect its tendency to break-down, but not always in the same direction for different varieties.

Storing promptly after picking frequently caused Jonathan, and usually Northwestern Greening, to be more susceptible to soggy break-down, while similar treatment of Grimes Golden, Wealthy, and Golden Delicious usually caused these varieties to be more resistant. Three seasons' work with Winter Banana showed a response similar to that of Jonathan.

With protracted delay before storing, i. e., from approximately 5 to 10 weeks at 50° F., Jonathan, Grimes Golden, Winter Banana, and Northwestern Greening exhibited marked resistance to soggy break-down. With similar treatment Golden Delicious developed more soggy break-down than the varieties just named, though it, too, tended to be resistant.

Results with picking-maturity and prestorage-delay experiments suggest that susceptibility to soggy break-down is associated with the stage of respiratory activity attained by the fruit at the time it is placed in storage.

Continuous movement of the storage-room atmosphere either over packed boxes of wrapped fruit or over fully exposed unwrapped fruit was unsatisfactory as a control measure for soggy break-down.

Studies on the periodic development of soggy break-down indicated initial and final dates of occurrence and a rather definite period of development.

Jonathan with 100-percent color in storage at 30° F. was more susceptible to soggy break-down than fruit of similar maturity having 25- to 50-percent color. Northwestern Greening of a full yellow color was more susceptible than distinctly green-colored fruit.

Of the varieties studied, Northwestern Greening was the only one in which large fruits were found to be more susceptible to soggy break-down than small fruits.

Apple varieties from the same orchards in different years, and from different orchards in the same year, exhibit marked differences in susceptibility to soggy break-down.

Investigations carried on for a number of years indicate that the most satisfactory method of controlling soggy break-down in all susceptible varieties under all the conditions tested, is to store apples at 36° F. rather than at lower temperatures.

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SOME PROPERTIES OF POTATO RUGOSE MOSAIC AND ITS COMPONENTS [1]

By DONALD FOLSOM, *plant pathologist*, and REINER BONDE, *associate plant pathologist, Maine Agricultural Experiment Station*

INTRODUCTION

Rugose mosaic was described by Schultz and Folsom (*20*, *21*,[2]) as one of several mosaic diseases of the potato (*Solanum tuberosum* L.) at a time when the idea that one host might have more than one virus disease of the mosaic type was somewhat new. Since then plants affected with the rugose mosaic disease have been shown to contain at least two viruses, one of which may be called the pure rugose mosaic

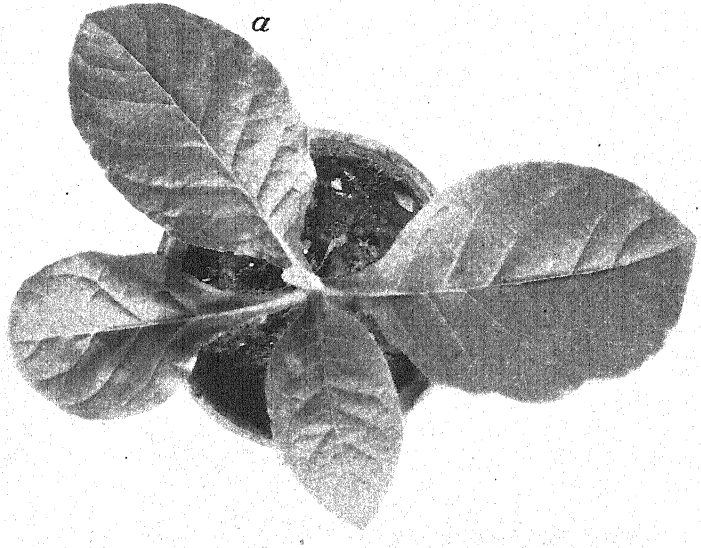


FIGURE 1.—Connecticut Broadleaf tobacco plant showing, in leaf *a*, faint vein clearing resulting from infection by pure rugose mosaic.

virus (fig. 1). The other, which has been designated the latent virus, is present in practically all American potatoes, as can be shown by inoculating plants of certain other species of Solanaceae. (See figs. 2 to 4 and also *3, p. 8; 9, fig. 5, B; 11, figs. 2, A and 3; 22, figs. 4 and 44; 17, fig. 9*). Vein banding, first described by E. M. Johnson (*8, pp. 299–300 and pl. X, fig. 1*) on Burley tobacco (*Nicotiana tabacum* L.), and later given as a symptom of rugose mosaic on Turkish tobacco

[1] Received for publication April 22, 1937; issued December 1937.
[2] Reference is made by number (italic) to Literature Cited, p. 782.

(*23, pls. 2, 3*), is not a symptom of pure rugose mosaic on Connecticut Broadleaf tobacco or Green Mountain potato. By some recent writers the pure rugose mosaic component of rugose mosaic is referred to as the "vein-banding virosis," sometimes being designated as "vein-banding virus" and sometimes as "vein banding." Rugose mosaic is said to be common and destructive in commercial potatoes in some regions (*13, p. 5*), and has been found by the writers attacking many new seedling varieties in breeding plots.

Property studies were begun by the writers with the twofold purpose of increasing the information on viroses, and developing simple physi-

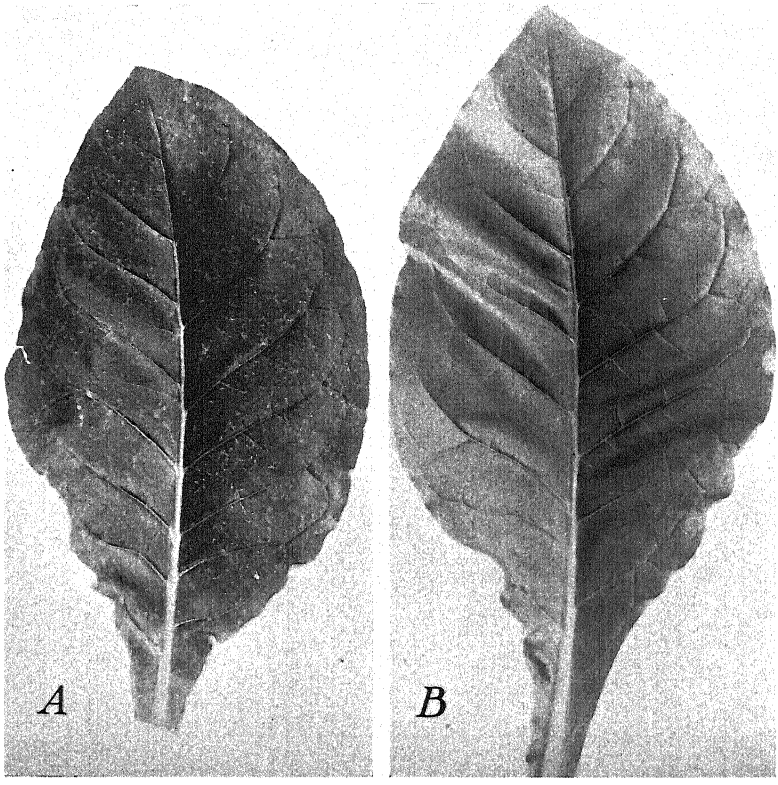


FIGURE 2.—*A*, Connecticut Broadleaf tobacco leaf from plant inoculated when young with juice from apparently healthy Green Mountain potato plants; *B*, uninoculated, healthy check. Photographed March 23, 1929.

cal and chemical tests for diagnosing potato viroses in local or imported lots of seed tubers. Several thousand plants in some hundreds of series were inoculated.

Although these property studies are concerned primarily with rugose mosaic and its components, comparisons were made between rugose mosaic and several other more or less similar virus diseases, such as tobacco spot necrosis, potato streak, and potato mild mosaic.

In potato plants of the Green Mountain variety in the normal Maine summer, the rugose mosaic disease is characterized by somewhat diffuse mottling, distinct dwarfing, rugosity premature death of

the plant, considerable reduction in yield of tubers, ease of infection by artificial methods, and slowness of natural translocation of the virus through infected plants and tubers.

A sequence of symptoms of rugose mosaic, typical of certain conditions, should be mentioned. Leaf-mutilation inoculation of young potato plants often produces, as the first symptoms in Green Mountain potatoes, in Maine, more necrosis than mottling. Streaking of veins and petioles and collapse and dropping of leaves occur at some distance above the inoculated leaves. The lower leaves, that is, the leaves below those that are subject to streaking and other forms of necrosis, show no symptoms, though their extract produces infection; the upper or youngest leaves merely develop mottling (*9, fig. 1, D*; *11, fig. 1, B*; *20, pl. 4, C*). Smith (*22, pp. 311–314, and fig. 50*), describes similar effects produced by virus Y. Apparently the rugose mosaic virus, upon becoming generally distributed through the plant, affects the leaves differently according to their age. Occasionally similar effects result from the delayed diffusion of the virus from the seed pieces of a tuber unit into growing plants (*21, pl. 8, C*), and hills and branches often display such effects as first symptoms.

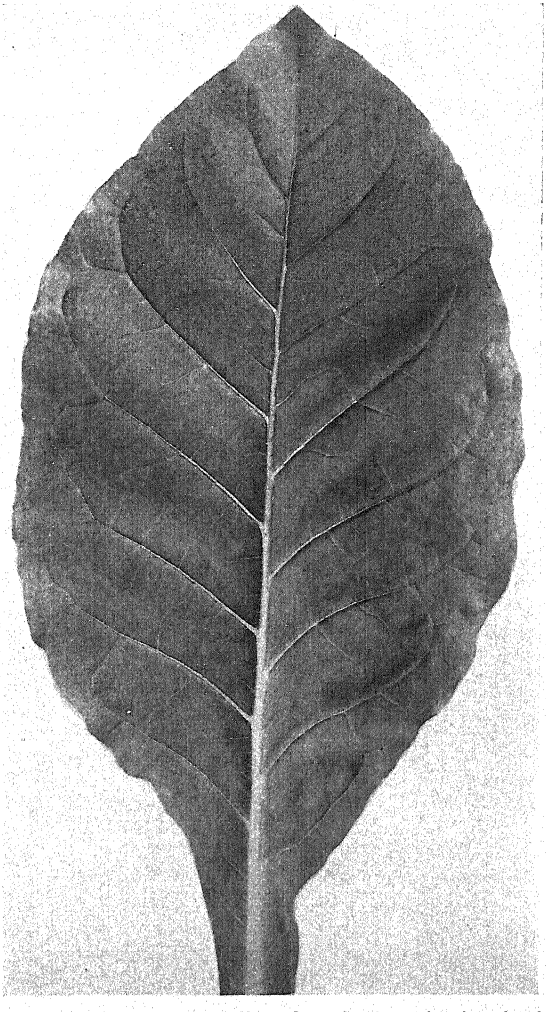


FIGURE 3.—Connecticut Broadleaf tobacco leaf from plant inoculated when young with juice from apparently healthy Green Mountain potato plants. For different effects of the same inoculation, on a nother plant, see figure 2*A*, and for healthy check see fig. 2, *B*. Photographed March 23, 1920.

A similar sequence of symptoms may occur in Connecticut Broadleaf tobacco. Vein clearing and necrosis (fig. 5) occur, as in the potato, as initial symptoms, in leaves above those inoculated. They sometimes occur in the lower or proximal end of one leaf, in the middle of the next higher leaf, and in the upper or distal end of the following leaf.

(See also the following: *9, fig. 6, B; 11, fig. 4, B; 22, fig. 3; 23, pl. 3.*) Inasmuch as the first part of a tobacco leaf to become full-formed is the distal, and the last part the proximal, leaf tissues which display the initial vein-clearing symptom are probably in approximately the same stage of development. Older tissues show no symptoms and younger tissues are merely mottled, or even apparently healthy, especially as the plant grows older.

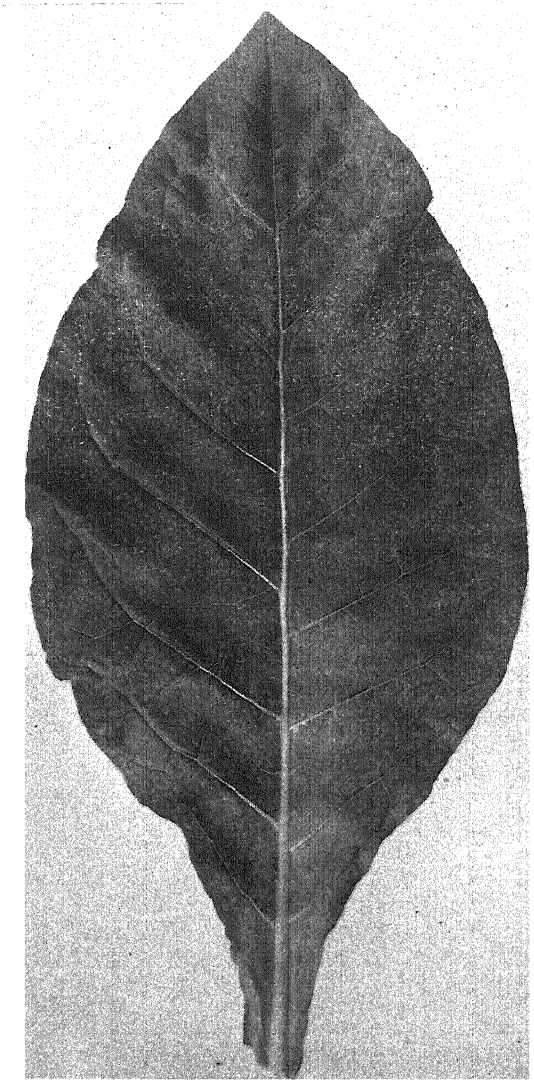


FIGURE 4.—Connecticut Broadleaf tobacco leaf of plant infected with latent mosaic from apparently healthy Green Mountain potatoes. Note the increasing severity of the symptoms from the distal and oldest end of the leaf, to the proximal end.

Such a sequence in Connecticut Broadleaf tobacco infected with pure rugose mosaic (free of the latent mosaic), is less conspicuous because there is less vein clearing and no necrosis (fig. 1).

It is not known whether the difference in severity of symptoms in leaves and parts of leaves of different ages in these tests was due to a difference in the amount of virus that developed or to a difference in reaction to similar amounts of virus. Acquired immunity could hardly be the explanation, though it might account for the absence of severe symptoms upon reinoculation of rugose mosaic potato plants that are diseased through virus perpetuation by the tubers. Price (*19*) has presented evidence of acquired immunity in tobacco propagated vegetatively after infection by a tobacco ring spot virus which was then found to be present in smaller amounts than in the leaves that had been inoculated artificially and showed the severest symptoms. However, in the case of rugose mosaic it is not the inoculated leaves

that show the severest symptoms, but rather some of those infected through systemic spread of the virus.

Potato extract often had an immediate and direct toxic effect upon tobacco leaves. This effect was more pronounced when rugose mosaic



FIGURE 5.—Connecticut Broadleaf tobacco plant showing vein clearing and rugosity in three younger leaves and collapse in one leaf, resulting from infection by rugose mosaic, a composite of pure rugose mosaic and latent mosaic.

was present in the potato plant that supplied the inoculum, but it could not be used as an indicator of rugose mosaic infection. It consisted of puckering, chlorosis or bleaching, and collapse and drying

of the interveinal tissue, all limited to the vicinity of the place of inoculation. It was prevented by filtration through a Berkefeld N candle even when the filtered extract caused rugose mosaic infection.

SOURCE OF INOCULUM AND CHOICE OF HOSTS

The usual source of inoculum was stock of the Green Mountain potato that had been perpetuated in tuber-unit disease-maintenance seed plots from the time that it was selected as typical of rugose mosaic potatoes. Tobacco plants, after infection by artificial inoculation, were used to some extent as a source of inoculum.

Inoculated plants were usually of the Green Mountain variety of potato, the Connecticut Broadleaf variety of tobacco, or jimsonweed (*Datura stramonium* L.). Tobacco was more susceptible to rugose mosaic than potato. Jimsonweed was not infected by the pure rugose mosaic component, but was ideal for testing properties of the latent mosaic component (*17, figs. 4–6*).

Less satisfactory preliminary results were obtained with plants of the Bonny Best variety of tomato (*Lycopersicon esculentum* Mill.), nightshade or wonderberry (*Solanum nigrum* L.), husk-tomato or groundcherry (*Physalis pubescens* L.), and apple-of-Peru (*Nicandra physalodes* (L.) Pers.).

Tomato, when infected with rugose mosaic as proved by inoculation back to potato, showed symptoms similar to those produced by the latent mosaic. Nightshade was mottled by the latent mosaic and, when rugose mosaic was present, showed more conspicuous mottling in addition to wrinkling and leaf dropping. Husk-tomato, when infected with rugose mosaic, was more wrinkled and rugose than when infected with the latent mosaic alone. Apple-of-Peru was dwarfed and blotched by the latent mosaic, and in addition was dwarfed, mottled, chlorotic, and wrinkled when infected with rugose mosaic. Plants of bean (*Phaseolus vulgaris* L.) were immune to both rugose and latent mosaic, or at least did not become infected when inoculated by the leaf-mutilation method.

METHODS OF INOCULATION

The leaf-mutilation method was most satisfactory for inoculating potato plants. The young leaves of plants about 10 cm high were rolled between the palms of the hands until they were considerably bruised, and they were then painted with the inoculum, in the form of expressed juice, by means of a cotton swab held in flamed tweezers. Sometimes the juice was applied with the fingers and sometimes it was poured onto the bruised leaves.

For inoculating tobacco and jimsonweed, the simplest method was satisfactory and consisted of using two new wooden pot stakes from those to be used in marking the plants of the series. One stake was used to support the leaf and the other to paint the inoculum upon the leaf surfaces (dorsal and ventral) and to bruise the leaf somewhat. Normally the three largest leaves were inoculated when the plant had six leaves a centimeter or more in length.

If inoculum was scarce, instead of the stake-painting method a diseased leaf was used, either as picked or after having been pulped in a mortar. The inoculum was held in flamed tweezers and the

inoculated leaf was supported with a flamed knife. A slice of tuber, used in this way, was not so effective as leaf tissue.

Other methods tried and found less satisfactory were painting with a soft brush, pricking with a needle, and cutting, pruning, or stabbing with a knife, each in addition to applying the inoculum. After a finger-painting method had been used, washing the fingers with soap did not make them noninfectious to jimsonweed, although it did to tobacco and husk-tomato. This confirms the conclusion of Fernow (*5*) as to the unreliability of soap and water as a virus disinfectant. Inoculation of stems and roots was less effective than inoculation of leaves.

In the greenhouse, where some potato plants and all plants of other species were inoculated, the temperature usually was held at 18° to 24° C. by thermostatic control.

When potato plants were inoculated, there was usually a control series that contained plants representing the respective tuber units to which the inoculated plants belonged. However, this proved to be an unnecessary precaution. Sometimes the tubers of inoculated plants were saved and planted and the second generation of plants observed, but when young plants were inoculated with rugose mosaic this was found to be unnecessary.

The control series developed very little uncontrolled or chance transmission, either in greenhouse or field.

INFECTIVITY OF THE VIRUS

INOCULUM FROM DIFFERENT ORGANS

Confirmation was attempted of a previous result obtained in testing different parts of the potato plant, namely, that the infectiousness of the rugose mosaic virus seemed to be correlated with the amount of chlorophyll present (*21*, *p. 507*). In one new experiment, extract from leaflets or from petioles and stems was completely effective on potato, while extract from colorless sprouts or seed tubers infected only one-sixth of the plants inoculated. In a second experiment, extract from the foliage was completely effective on potato, extract from colorless sprouts or seed tubers infected five-twelfths of the inoculated plants, and extract from roots infected only one-sixth. When seed tubers were greened by exposure to light, the amount of greening was not correlated with variations in infectiousness of the extract. However, clarifying extract from foliage by appropriate filtration sometimes reduced infectiousness.

McKinney (*15*, *p. 35*) found the concentration of virus in mosaic tobacco to be much greater in leaf extract than in stem extract. In potatoes affected by spot necrosis, which is about the same as rugose mosaic, Valleau and E. M. Johnson (*23*, *p. 484*) found colorless sprouts noninfectious while the green leaves were highly infectious. Matz (*16*) reports lower rates of infectiousness by sugarcane mosaic from extract of white tissues than green tissues of the same shoots.

AGE OF PLANT SUPPLYING THE INOCULUM

Inoculum was secured July 21 to 31, about midway between planting and harvesting, from rugose mosaic potato plants grown in the field. Such inoculum was more infective than inoculum secured previously or subsequently. Further, inoculum from young plants infected all

inoculated plants, and symptoms appeared in 14 to 20 days. With inoculum from leaves midway along the stem of old plants 4 feet long and dying, 80 percent of the inoculated plants were infected and symptoms first appeared in 31 days. Inoculum from dry leaves of a plant dying 2 days previously from old age produced no infection.

In several comparisons, the age of tobacco plants with rugose mosaic had very slight if any effect upon the infectiousness of their extract when it was diluted with enough distilled water to reduce the proportion of extract to 10, 1, and 0.1 percent of the inoculum.

Diseased tobacco leaves dried for 8 months were not infectious.

AGING OF INOCULUM IN VITRO

Usually extract, to be used as inoculum, was obtained by grinding the plants through a food chopper, collecting the pulp in gauze, and squeezing out the sap by hand into a glass or porcelain container. The extract was then stirred well and divided into equal portions, and these were allowed to stand, or "aged," in vitro until used for inoculation. The important results of several illustrative comparisons are given in table 1.

Apparently aging in vitro often increased the infectiousness of rugose mosaic inoculum up to 4, 6, or 8 hours of aging, depending upon the series, while further aging decreased infectivity until it was lost. This loss in infectiousness required more aging at 5° C. than at 17° to 30°. At 30° the loss developed earlier with potato than with tobacco as the inoculated plant, occurring respectively before 5 days and after 10 days. At about 23° for potato, infectiveness was lost in 3 days by one inoculum, in 4 days by another, and in 5 days by others, while being retained by one for at least 7 days. At 15° for tobacco, infectiveness was lost in 7 days.

Increase in infectiousness of extract after a certain amount of aging in vitro has been reported for tobacco mosaic by Elmer (*4*), Olitsky and Forsbeck (*18*), and McKinney (*14, p. 8*), and for curly top by Bennett (*1, table 15, second, ninth, and last sources*).

Mosaic virus of the latent type sometimes resisted aging slightly better than that of rugose mosaic, and aging was not an effective means of freeing pure rugose mosaic from latent.

TABLE 1.—*Effect of aging, at normal and at low temperatures (° C.), upon the infectivity of rugose and latent mosaic extracts from potato and tobacco*

Series [1]	Virus	Source of inoculum	Plants inoculated	Aging of inoculum	Results
1	Rugose mosaic	Potato	Potato	None	All hills infected, most completely and severely with 4 hours' aging. Symptoms appearing later with 24–30 hours' aging.
				4 hours	
				8 hours	
				24 hours	
				30 hours	
2	do	do	do	None	All hills infected, most completely and severely with 8 hours' aging. Delay of appearance of symptoms, with 24–30 hours' aging, greater than in series 1.
				4 hours [2]	
				8 hours [2]	
				24 hours [2]	
				30 hours [2]	
3	do	do	do	None	All hills infected. Increased infection. Decreased infection progressively.
				8 hours	
				24 hours	
				2 days	
				7 days	

[1] Series 1–5 and 8 were in the field, and the rest in the greenhouse, at about 23° C.
[2] In sunlight for at least 8 hours, for comparison with series 1, kept in the dark.

TABLE 1.—*Effect of aging, at normal and at low temperatures (° C.), upon the infectivity of rugose and latent mosaic extracts from potato and tobacco*—Contd.

Series	Virus	Source of inoculum	Plants inoculated	Aging of inoculum	Results
4	Rugoes mosaic	Potato	Potato	None	No difference; all hills infected.
				2 days	
				3 days	Decreased infection.
				4 days	No infection.
				5 days	
				8.5 days	
				11 days	
5	do	do	do	None	All hills infected.
				1 hour	Most infection at 4 and 6 hours.
				2 hours	
				4 hours	
				6 hours	
				8 hours	
				3 days	Infection with aging at 5° but not at 17°, 23°, and 20°.
				5 days	
				7 days	No infection with aging at any temperature.
				8 days	
				11 days	
				17 days	
				38 days	
6	do	do	do	5 days	Infection with aging at 5° but not at 30°.
				13 days	No infection.
7	do	do	Tobacco	5 days	Infection decreased with aging at 30° as compared with 5°.
				7 days	
				10 days	
8	do	do	Potato	None	Infection increased up to 4–6 hours, decreased in 8 hours, lost at 5 days.
				2 hours	
				4 hours	
				6 hours	
				8 hours	
				5 days	
9	do	do	do	17 days	Infection with aging at −6°.
				5 days	No infection with aging at 20°.
				7 days	
				10 days	
10	do	do	Tobacco	8 days	88 percent infected with aging at −18° in frozen shoot.
				17 days	No infection with aging at 5° but all infected with aging at −7°.
				20 days	33 percent infected with aging at −7° in frozen shoot.
				36 days	No infection with aging at −7° in frozen shoot.
11	Latent mosaic	do	Jimsonweed	17 days	All infected with aging at −7° in frozen shoot.
[3] 12	Rugose mosaic	do	Tobacco	None	All infected, most with symptoms in 13 days.
				1 hour	
				2 hours	
				3 hours	
				4 hours	
				5 hours	
				6 hours	
				7 hours	All infected, minority with symptoms in 13 days.
				8 hours	All infected, none with symptoms until after 13 days.
				1 day	
				2 days	Majority infected.
				3 days	
				4 days	
				5 days	
				6 days	Minority infected.
				7 days	None infected.
				8 days	
				9 days	
				10 days	

[3] Aging at about 15° C.

Aging in potato extract in vitro usually inactivated rugose mosaic inoculum within a few days, according to James Johnson (*9, table II*) and James Johnson and Grant (*10, table 7*), both authorities finding the latent type persisting somewhat longer. Similar results are reported

by Koch (*11, table 8*).[3] Van der Meer (*17, table 4*) reported persistence of the latent mosaic for less than 6 days in one test, and for nearly 3 weeks in others.

TEMPERATURE

A common method of determining the so-called thermal death point of a virus is to immerse a tubeful of inoculum for a certain length of time in water maintained at a constant temperature. This requires frequent readjustment of the water bath, or the use of several baths, if a comparison is to be made of the effects of different temperatures on parts of the same inoculum. The writers heated a water bath gradually, removing and cooling the sublots of inoculum as the rising temperature, registered by a thermometer in the inoculum, reached various points. Many such comparisons were made but need not be given here. The results of several typical comparisons are shown in table 2.

TABLE 2.—*Effect of high temperatures on infectivity of rugose mosaic inoculum from potato*

Series	Plants inoculated	Temperature regulation, ° C.		Results
[1] 1	Potato	Raised about 10° every 8 minutes to.	50	Complete infection.
			60	Little infection.
			70	No infection.
			80	Do.
2	Tobacco	Raised about 10° every 4 minutes to.	50	Complete infection.
			55	Do.
			60	Do.
			65	No infection.
			70	Do.
			75	Do.
			80	Do.
			85	Do.
			90	Do.
3	Potato	Raised to	45	Complete infection.
			50	Do.
			55	Do.
			60	No infection.
			65	Do.
			70	Do.
4	Tobacco	Raised to	60	All latent mosaic.
			65	Do.
			80	80 percent latent mosaic.
			95	No infection.
5	do	Raised to	40	Complete infection.
			45	Do.
			50	Do.
			55	83 percent infected.
			60	Only latent mosaic.
			65	Do.
			70	Do.

[1] Series 1 was in the field; the rest were in the greenhouse.

Rugose mosaic virus usually was inactivated by raising the temperature to 60° or 65° C., or by holding the temperature for 10 to 15 minutes at 55°. Even with conditions apparently the same from series to series, the results were not alike. A higher temperature was required for tobacco extract used on tobacco than for transfers from potato to potato, potato to tobacco, or tobacco to potato. Exposures to temperatures just below those able to inactivate increased the time required for the first symptoms to appear and decreased the percentage

[3] He refers to vein banding, ring spot, and mottle; in a later publication he and Johnson state that "For all practical purposes, potato 'rugose mosaic' is due to the vein-banding virus" (*12, p. 42*), though actually including mottle or ring spot in addition (*p. 41*).

of plants infected; no change in the nature of the symptoms was observed. The results with pure rugose mosaic were much the same as with rugose. The latent mosaic present in rugose mosaic had a higher limit of tolerance, sometimes persisting to 90°.

The thermal death point of rugose mosaic virus has been found by others to lie between 60° and 65° (*9, p. 11*), 55° and 60° (*10, table 3*), and 58° and 60° C. (*11, table 7*), all at 10-minute exposures to constant temperature. The corresponding limits of the latent mosaic were about 70° (*9, p. 11; 10, table 3*), 65° to 70° (*11, table 7*), and 70° to 75° (*17, table 5*).

DILUTION

Extract from diseased plants was diluted to various degrees with water of different kinds and with juice from healthy plants, with a resulting reduction in infectiousness, at least when a certain point of dilution had been reached. The results of the tests with diluted extracts, from different sources transferred to one host or another, are given in part in table 3. These results are representative of a large mass of data which show in general that infectiousness was lost when the proportion of extract in the inoculum had been reduced to between 10 and 1 percent by distilled water (in transmission tests from potato to potato in the greenhouse); to between 1.0 and 0.5 percent by healthy potato juice (potato to potato in the field); and to between 1.0 and 0.1 percent by soft lake water and distilled water (potato to tobacco in the greenhouse). Some infectiousness persisted at 1.0 to 0.1 percent in series diluted with hard river and well water (potato to potato in the field) and with distilled water (potato to tobacco and tobacco to tobacco in the greenhouse). As the limit was approached, there was a decrease in the percentage of plants infected by inoculation and an increase in the time required to bring out the first symptoms.

The latent mosaic was somewhat more persistent at the dilution inactivation point of rugose mosaic.

As compared with the above inactivation point of about 0.1 percent, or about one one-thousandth, it is interesting to note that the inactivation points reported by others for rugose mosaic are about one one-thousandths (*9, p. 11*) and one five-thousandths to one ten-thousandths (*11, table 9*), and for the latent mosaic, one ten-thousandths (*9, p. 11*), one ten-thousandths to one one-millionths (*11, table 9*), and about one one-hundred-thousandths (*17, table 6*).

The greater inactivating effect of healthy potato juice, as compared with water in series 2 and 3 of table 3, upon rugose mosaic, is similar to that reported by McKinney (*14, table 5*) for tobacco and cucumber extracts.

The inactivation of tobacco mosaic virus by pokeweed (*Phytolacca decandra* L.) juice in vitro (*14, p. 9*) was found to hold also for rugose but not for latent mosaic, and pokeweed juice painted onto tobacco leaves several days before inoculation with rugose mosaic reduced infection below that obtained on water-painted checks.

TABLE 3.—*Effect of dilution upon infectivity of rugose mosaic virus from potato*

Series [1]	Plants inoculated	Diluent	Percent extract in dilution	Results
1	Potato	River water	100	All infected.
			50	Infection of all, but progressively less severe.
			20	
			10	
			5	88 percent infected.
			2	20 percent infected.
			1	10 percent infected.
2	do	Well water	50	All infected.
			20	
			10	
			5	All infected; majority incompletely.
			2	90 percent infected.
			1	70 percent infected.
			.5	44 percent infected.
			.2	20 percent infected.
			.1	22 percent infected.
3	do	Healthy potato extract	50	90 percent infected.
			20	40 percent infected.
			10	30 percent infected.
			5	20 percent infected.
			2	33 percent infected.
			1	11 percent infected.
			.5	None infected.
			.2	
4	do	Distilled water	50	All infected.
			10	
			1	None infected.
			.1	
			.01	
5	Tobacco	do	50	All infected.
			10	
			1	83 percent infected.
			.1	None infected.
			.01	
6	do	do	5	60 percent infected.
			1	None infected.
			.5	40 percent infected.
			.1	None infected.
			.05	
			.01	
			.005	
			.001	

[1] Series 1–3 were in the field; the rest were in the greenhouse. Series 2 and 3 were kept under similar conditions.

FILTRATION

Rugose mosaic potato extract filtered through Berkefeld N and V candles infected a small percentage of inoculated tobacco plants, but usually no potato plants. The percentage of infection was not increased by previously clarifying the extract by filtration through paper that had become coated with the gelatinous mass of crushed plant tissue, or filter slime. Neither was it increased by filtration under 80 to 90 pounds' pressure per square inch in a Hill pressure apparatus. Here the unfiltered sediment in the rubber bag enlosing the candle retained its full infectiousness, although exposed to the pressure indicated. (More than 75,000 pounds' pressure was required to inactivate mosaic tobacco extract (7).) Clarification did not inactivate the extract, although sometimes it reduced infectiousness slightly. Of 10 potato plants inoculated in the field with extract filtered under air pressure through a Chamberland B filter, 1 became diseased, possibly through uncontrolled natural infection. Of 18 potato plants inoculated in the greenhouse with clarified extract filtered through a Berkefeld V candle with suction that reduced the pressure to about one-fifth of an atmosphere, 1 became diseased. Filtration through a Berkefeld V candle had little effect on the infectiousness of latent mosaic extract.

CHEMICALS

Each chemical to be tested was dissolved in water in a series of different concentrations in equal amounts of solvent. Then the solution at each concentration was combined with an equal amount of fresh extract. For example, to get a 5-percent concentration, a 10-percent solution of the chemical was made up and added to an equal amount of the fresh extract. Thus the resulting mixture contained a 50-percent concentration of the extract and half the concentration of chemical in the original solution. The mixing was followed by inoculation within about 10 minutes. The order of mixing and inoculation was according to decreasing concentration of the chemical. The chemicals tested are representative of several important types of compounds, and are readily available, easily prepared for use, and for the most part, of recognized value as disinfectants or fungicides. The results are given in table 4.

The lethal point of hydrochloric acid for rugose mosaic virus varied with conditions. It was between 0.07 and 0.2 percent in series 4 (in transmission tests from potato to potato in the field); between 0.2 and 0.5 in series 11 (potato to potato in the greenhouse), in series 12 and 13 (potato to tobacco in the greenhouse), and in series 14 (tobacco to potato in the greenhouse); and beyond 0.5 percent in series 15 (tobacco to tobacco in the greenhouse). However, the closeness of results probably was more remarkable, considering the different conditions, than the variation. The lethal point of sodium hydrate also varied. In the order of decreasing concentration needed to inactivate, the tested chemicals and their inactivation points were as follows: Ethyl alcohol (C_2H_5OH), over 50 percent; sodium chloride (NaCl), about 5 percent or 1 to 20; formaldehyde (HCHO), about 0.5 percent or 1 to 200; hydrochloric acid (HCl) and sodium hydrate (NaOH), respectively, about 0.2 percent or 1 to 500; and copper sulphate ($CuSO_4$) and sulphuric acid cleaning fluid, respectively, about 0.1 percent or 1 to 1,000. Prolonging the duration of exposure to the chemical to an hour did not shift the point much (series 17 versus 18).

TABLE 4.- *-Effects of chemicals upon infectivity of rugose mosaic virus from potato and tobacco*

Series [1]	Source of inoculum	Plants inoculated	Chemical	Percent of chemical	Results
1	Potato	Potato	C_2H_5OH	5 and less	All infected.
				10	Mostly infected.
				25 and 50	Some infected.
2	do	do	C_2H_5OH [2]	As in series 1	As in series 1.
3	do	do	HCHO [3]	2 and more	No infection.
4	do	do	HCl [4]	0.01	All infected.
				0.07	20 percent infected.
				0.2 and more	No infection.
5	do	do	NaOH	0.1	50 percent infected.
				0.2 and more	No infection.
6	do	do	NaCl	0.1 to 1	All infected.
				2	50 percent infected.
				5	10 percent infected.
7	do	do	HCHO	0.004 to 0.2	80 to 100 percent infected.
				0.3	50 percent infected.
				0.7 and more	No infection.

[1] Series 1-8 were in the field; the rest were in the greenhouse.
[2] Commercial "denatured" material was used in series 2.
[3] Percentages calculated on basis of actual HCHO; thus percent in terms of the 40-percent solution used would be 5 and more.
[4] Percentages calculated on the basis of actual HCl; thus each is only about two-fifths of concentration of the c. p. material used, with specific gravity 1.19.

TABLE 4.—*Effects of chemicals upon infectivity of rugose mosaic virus from potato and tobacco*—Continued

Series	Source of inoculum	Plants inoculated	Chemical	Percent of chemical	Results
8	Potato	Potato	$CuSO_4$	0.1	80 percent infected.
				0.2	No infection.
				1 and more	Do.
9	do	Tobacco	C_2H_5OH	20 and less	All infected.
				50	13 percent infected.
10	do	do	HCHO	0.2	All infected.
				0.5	40 percent infected.
				1 and more	No infection.
11	do	Potato	HCl	0.1 to 0.2	40 percent infected.
				0.5	No infection.
12	do	Tobacco	HCl	0.1	All infected.
				0.2	20 percent infected.
				0.5	No infection.
13	do	do	HCl	0.1	33 percent infected.
				0.2	17 percent infected.
				0.5	No infection.
14	Tobacco	Potato	HCl	0.1 to 0.2	All infected.
				0.5	No infection.
15	do	Tobacco	HCl	0.1	60 percent infected.
				0.2	81 percent infected.
				0.5	33 percent infected.
16	Potato	do	NaOH	0.05 to 0.1	All infected.
				0.2	No infection.
17	do	do	NaOH	0.05	All infected.
				0.1	40 percent infected.
				0.2	60 percent infected.
[5] 18	do	do	NaOH	0.05 to 0.1	All infected.
				0.2	No infection.
19	Tobacco	do	do	0.05 to 0.1	All infected.
				0.2	80 percent infected.
20	Potato	do	NaCl	1	All infected.
				5	14 percent infected.
				10	No infection.
21	do	do	$CuSO_4$	0.1	17 percent infected.
				0.2	No infection.
				0.5 to 1	Plants killed.
22	do	do	Cleaning fluid [6]	0.05 to 0.1	No infection except by latent component.
				0.25 to 2.5	No infection.
[7] 23	do	Jimson weed	do	0.05 to 0.1	Latent mosaic.
				0.25 to 2.5	No infection.
24	do	Tobacco	do	0.01 to 0.1	All infected.
				1	No infection except by latent component.
25	do	do	NaOH	0.1	20 percent infected.
				0.2	All infected.
				0.5	None infected.
				1	

[5] Same inoculum as for series 17 except mixed 1 hour before being used.
[6] Made up with sulphuric acid and potassium bichromate.
[7] Series 22 and 23 were under the same conditions.

The lethal point for the latent component did not differ much from that for rugose mosaic except for greater resistance of the latent to the toxic effects of formaldehyde and the cleaning fluid.

James Johnson (*9, p. 12*) and Koch (*11, table 10*) report similar results with alcohol and HCHO. Van der Meer also found alcohol a weak inactivator (*17, table 7*). Freeman (*6*) found that from rugose mosaic only the latent mosaic component could be recovered at pH 4 to 5.5 and at 9.7; from pH 5.6 to 7.6 there was no inactivation, and at pH 3.6 or lower not even the latent component could be recovered.

COMPARISON OF RUGOSE MOSAIC WITH OTHER MOSAICS

MILD, CRINKLE, AND LEAF-ROLLING POTATO MOSAICS

Leaf-mutilation inoculation of bean plants with potato mild mosaic and leaf-rolling mosaic extract produced no symptoms. Stake-painting inoculation of potato plants with potato mild mosaic extract did

not always infect and, when it did, produced late and therefore unsatisfactory symptoms. Similarly, unsatisfactory symptoms resulted on potato with the potato crinkle and leaf-rolling mosaics.

TOBACCO MOSAIC

Dried leaves from the laboratory of B. M. Duggar in St. Louis, Mo., from experiments of F. M. Blodgett at Cornell University, Ithaca, N. Y., and from a field in Connecticut, were used as the sources of tobacco mosaic inoculum which gave infection on potato plants. The symptoms were not those of a mosaic, but were rather like those of streak with some suggestion of blackleg, and included necrotic spotting and streaking of the leaves, streaking of the stem, leaf dropping, wilting, and early death of the plant. These results agree with those reported by Fernow (*5, table 21 and pl. VII*) and Blodgett (*2*). The St. Louis strain, used on jimsonweed, caused a slight amount of spot and streak necrosis at first but soon became masked, though still present in virulent form as was shown by transfer back to tobacco.

STREAK

Although causing no mottling of Green Mountain potatoes, streak produced mosaic on tomato and Spaulding Rose potato plants, and when on tobacco, nightshade, and apple-of-Peru, was not to be distinguished by symptoms from rugose mosaic. Jimsonweed, immune to rugose mosaic, also was immune to streak, and the raising of the temperature to 60° C. inactivated streak in vitro. The inactivating effects of pokeweed juice on rugose mosaic were duplicated in tests repeated with streak. These inoculation studies suggest that the streak virus is very similar to the virus of rugose mosaic. A tomato plant inoculated on February 3, 1927, and used for successful return inoculation to potato, was still vigorous on October 17, 1928 (fig. 6).

TOBACCO SPOT NECROSIS

Tobacco leaves infected with spot necrosis were kindly supplied the writers by James Johnson, of the Wisconsin Agricultural Experiment Station, and in Maine they produced a disease on greenhouse potato and tobacco very similar to rugose mosaic but somewhat more virulent and infectious. A second supply from Johnson consisted of both tobacco and potato spot necrosis leaves which were used on greenhouse tobacco in comparison with inoculum from Maine rugose mosaic potato. Here the tobacco-leaf inoculum was more infective and had a shorter incubation period than the other two inocula. However, when the three newly infected tobacco series were compared by inoculating potatoes in the field, the strain received from Johnson in the potato leaves was the only one that gave current-season symptoms, and it was less infectious than inoculum from field-grown rugose mosaic potato plants. Such effects of host upon infectivity are similar to those reported by Johnson (*9, table III*).

James Johnson stated (*9, p. 4*), in 1929, that after repeated attempts at correlation, he had concluded that his spot necrosis was identical with the rugose mosaic of Schultz and Folsom; that is, with the rugose mosaic used in the present comparisons. He also explained (*9, p. 23*) that "in previous literature from the Wisconsin laboratory

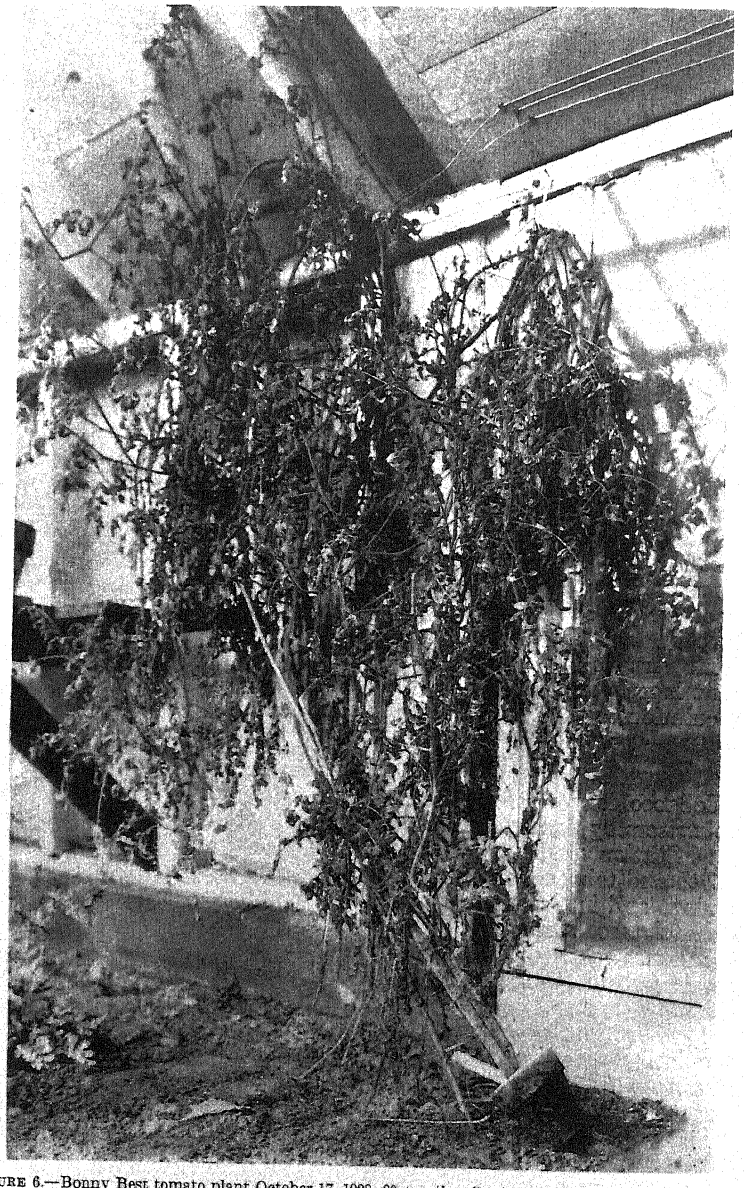


FIGURE 6.—Bonny Best tomato plant October 17, 1928, 20 months after infection with potato streak from Spaulding Rose potato plants and 18 months after back-inoculation to Green Mountain potato plants that resulted in the death of the plants from streak. This photograph was taken shortly after a second virus also a mosaic, had spread through the plant, and shortly before the plant succumbed.

referring to 'rugose mosaic' of Triumph potatoes, this name should read 'crinkle mosaic'."

ATTENUATION AND INCREASE IN VIRULENCE

The idea that a virus may increase in virulence is met many times in the study of mosaics, including those of potato. A tuber-propagated host would seem to be promising material from which to obtain evidence. In the writers' experience, an apparent increase in virulence such, for example, as mild mosaic apparently becoming rugose mosaic, has occurred chiefly in proximity to plants already showing the more severe disease, under conditions favorable to transmission, or, of course, in plants inoculated experimentally with the more virulent type of the virus. Isolation in effective insect cages has prevented an increase of virulence of mosaic, even in stocks perpetuated for about 20 years.

In greenhouse studies in Maine efforts to increase the virulence of latent mosaic until it resembled spot necrosis or rugose mosaic, by passage through as many as eight successive series of tobacco plants, have failed to bring about any change from one mosaic to the other. The symptoms of both kinds of mosaic vary. Leaf contact is sometimes sufficient to transmit either mosaic. The general tendency in virology is toward longer lists of viroses for various hosts and longer lists of means of transmission of the respective viroses, so that the chance of uncontrolled contamination by a severer type, through an unsuspected agency, seems more reasonable than a spontaneous sudden increase in virulence. In a discussion of this problem, Johnson (*9, pp. 18–21*) states that "the mottle form from healthy potatoes cannot ordinarily be changed to spot necrosis at will," and points out the possibility of accidental transmission of spot necrosis as the cause of a sudden increase in virulence.

Apparent attenuation of a virus often may be reasonably explained on the basis of (1) the elimination of one or more components of a composite or multiple virosis, (2) a decrease in the severity of symptoms after the first have developed or (3) a decrease in the severity of symptoms with certain changes in environmental conditions or with increasing age of the host plant.

SUMMARY

Potato rugose mosaic is a composite mosaic which includes pure rugose mosaic, or the vein-banding mosaic, and latent potato mosaic When contracted by a partly grown potato or tobacco plant, rugose mosaic often affects leaves and leaf parts differently according to their age; some become necrotic; older ones show no symptoms and younger ones develop mottling. Rugose mosaic aggravated a toxic effect of potato extract upon tobacco; the effect was eliminated by Berkefeld-candle filtration.

Rugose and latent mosaic were studied better on potato, tobacco, and jimsonweed than on tomato and several other species of the same family; bean was immune.

The most satisfactory method for inoculating potato plants was the leaf-mutilation method, and the best for inoculating tobacco and jimsonweed was a stake-painting method.

Extract from green shoots was more infectious than that from colorless sprouts, seed tubers, and roots of rugose mosaic potato plants, and was sometimes made less infectious by clarification.

With rugose mosaic, the age of potato plants or their parts had more effect than the age of tobacco plants in determining the infectiveness of their extracts. Drying of leaves soon eliminated the infectivity.

Aging in vitro for several hours progressively increased the infectiousness of rugose mosaic extract; further aging progressively reduced it, and under certain conditions the inactivation-point was reached in a few days. Infectiveness was inhibited later at low temperatures (5° C.), and later for tobacco than for potato as the inoculated host. The latent mosaic virus sometimes resisted aging longer than the pure rugose mosaic virus.

Rugose mosaic extract usually was inactivated when the temperature was raised to 60° or 65° C., or when the temperature was held for 10 minutes at 55°, but the thermal death-point varied with the species involved in the transfer and also with the series even when conditions apparently were similar. Pure rugose mosaic acted like the composite virus, but the latent component had a higher limit of tolerance.

Rugose mosaic extract became inactivated at about 1 to 0.1 percent upon dilution with water. Healthy potato juice had a slightly greater inactivating effect than water. Latent mosaic was somewhat more persistent than rugose. Pokeweed juice inactivated rugose mosaic extract but not latent mosaic.

The virulence of rugose mosaic virus was reduced considerably but not eliminated by filtering the extract through bacteria-retaining candles; the latent mosaic virus was affected only a little by the process. Neither 80 pounds' pressure nor clarification reduced the infectiousness of rugose mosaic extract more than a slight amount.

Latent mosaic was more resistant to formaldehyde and a sulphuric-acid cleaning fluid than rugose, but was similar in its response to other chemicals. The lethal point of HCl for rugose mosaic varied with conditions, within the range of about 0.07 to over 0.5 percent. To inactivate, the strength of ethyl alcohol had to be greater than 50 percent; of NaCl, about 5 percent; of HCHO, about 0.5 percent; of HCl and NaOH, about 0.2 percent; and of $CuSO_4$ and the cleaning fluid, about 0.1 percent.

Preliminary comparisons between mosaics on potato and other plants showed that the methods used for these property studies of rugose mosaic are unsatisfactory for mild, crinkle, and leaf-rolling mosaics of potato. Tobacco mosaic can infect Green Mountain potato plants. Potato streak and tobacco spot necrosis both resemble rugose mosaic.

Latent mosaic was not increased in virulence by eight successive passages through tobacco plants.

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RESPONSE OF OAT VARIETIES TO DIFFERENT FERTILITY LEVELS [1]

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INTRODUCTION

A 3-year rotation of corn, oats, and winter wheat has been grown at four fertility levels at the Ohio Agricultural Experiment Station at Wooster. Data were collected for all three crops, and those for corn and winter wheat have been published.[3] The present paper is concerned with the oat crop in this rotation.

The literature has been reviewed in the papers mentioned above, and repetition seems unnecessary. Suffice it to say that differential response of varieties to fertility level in many of our common crops has proved significant, and that this interaction deserves careful study.

OUTLINE OF EXPERIMENT

The 3-year rotation of corn, oats, and winter wheat was laid out on three fields of Canfield silt loam of low fertility. The higher fertility levels were obtained by adding one, two, and four increments of fertilizing materials. Actual applications are given in table 1. The oats received no direct applications of fertilizers.

TABLE 1.—*Plot treatments at the 4 fertility levels used in 3-year rotation tests with corn, oats, and winter wheat*

Fertility level	Treatment used for crop indicated		
	Corn	Oats	Wheat
A	None	None	None.
B	4 tons manure; 100 pounds 0-16-0 broadcast and 100 pounds 4-12-4 in hill.	None	200 pounds 2-14-4 in fall and 50 pounds nitrate of soda in spring.
C	8 tons manure; 200 pounds 0-16-0 broadcast and 200 pounds 4-12-4 in hill.	None	400 pounds 2-14-4 in fall and 100 pounds nitrate of soda in spring.
D	16 tons manure; 400 pounds 0-16-0 broadcast and 400 pounds 4-12-4 in hill.	None	800 pounds 2-14-4 in fall and 200 pounds nitrate of soda in spring.

The four levels were set up on adjacent strips in each field and the varieties sown across them. Oats were planted in triplicate; the size of plot was 5⅓ by 35 feet (0.00427 acre). The order of varieties in each replication was the same in 1929, random in the other 3 years. In all cases each variety was sown in a continuous strip across the fertility levels; thus the order of plots was identical on each level.

[1] Received for publication June 1, 1937; issued December 1937. Contribution from the Department of Agronomy, Ohio Agricultural Experiment Station.

[2] The writers wish to acknowledge the help of G. H. Stringfield, who, with R. M. Salter, set up the experiment. He selected the varieties and collected the data for the early years, and has offered helpful suggestions in the preparation of the manuscript.

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PRESENTATION OF DATA

Seventeen varieties of oats were grown for four seasons, 1929 to 1932, inclusive. Table 2 gives the grain yield, straw yield, and bushel weight for each variety-level-season unit. In figure 1 the average performance of all 17 varieties in each season has been plotted.

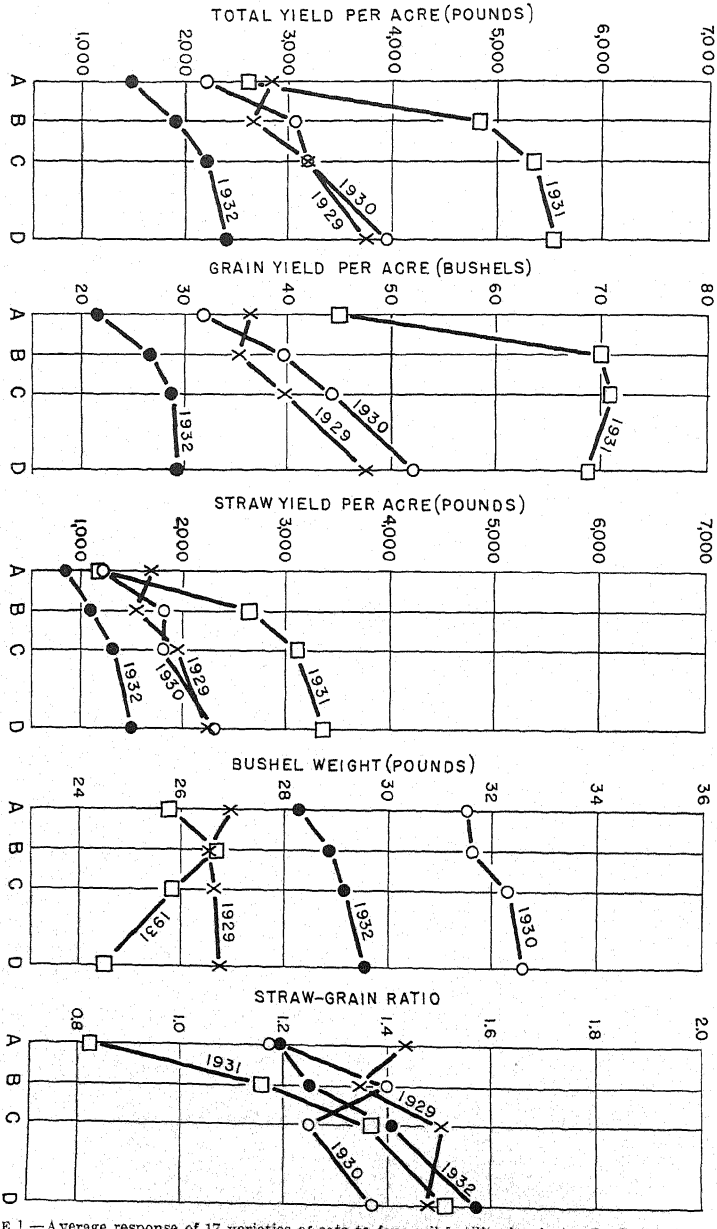


FIGURE 1.—Average response of 17 varieties of oats to four soil-fertility levels (A, B, C, D) during four seasons

TABLE 2.—*Yield per acre and weight per bushel of 17 varieties of oats grown at 4 levels of soil fertility, 1929–32*

Variety	Level	1929			1930			1931			1932			Average		
		Grain	Straw	Weight per bushel	Grain	Straw	Weight per bushel	Grain	Straw	Weight per bushel	Grain	Straw	Weight per bushel	Grain	Straw	Weight per bushel
		Bushels	*Pounds*	*Pounds*	*Bushels*	*Pounds*	*Pounds*	*Bushels*	*Pounds*	*Pounds*	*Bushels*	*Pounds*	*Pounds*	*Bushels*	*Pounds*	*Pounds*
Burt	A	32.9	1,444	26.2	26.6	852	30.4	42.2	1,220	25.0	21.5	860	28.9	30.8	1,094	27.6
	B	32.3	1,230	25.6	36.8	1,178	30.1	60.9	2,100	27.1	24.5	1,037	29.0	38.6	1,386	28.0
	C	36.0	1,815	26.0	42.7	1,365	31.8	71.1	3,240	26.7	27.8	1,399	28.5	44.4	1,955	28.3
	D	43.3	1,971	25.7	47.8	1,529	31.9	63.8	4,012	25.3	25.7	1,465	29.0	45.2	2,244	28.0
Albion	A	30.5	1,132	26.9	23.3	747	30.1	42.4	1,220	25.7	20.6	662	27.1	29.2	940	27.5
	B	29.9	1,073	26.9	27.7	887	31.6	69.7	2,360	26.5	26.8	855	28.8	38.5	1,294	28.5
	C	37.8	1,444	26.4	32.1	1,027	31.7	63.9	2,480	24.8	29.4	1,049	28.5	40.8	1,500	27.9
	D	43.9	1,717	26.9	40.5	1,295	32.1	61.8	1,980	24.8	29.4	1,161	28.6	43.9	1,538	28.1
Richland	A	33.5	1,269	27.4	22.6	755	30.4	40.4	720	25.8	20.8	759	27.3	29.3	876	27.7
	B	34.2	1,249	27.2	31.1	1,260	31.2	62.4	1,980	26.4	24.5	802	28.3	38.1	1,323	28.3
	C	37.2	1,464	27.0	34.3	1,470	31.1	63.0	2,500	24.8	25.7	913	28.3	40.1	1,587	27.8
	D	42.7	1,756	27.0	45.2	1,742	32.2	69.1	3,260	23.5	28.8	1,232	29.1	46.5	1,998	28.0
Fulghum	A	28.7	1,425	28.2	25.0	1,066	31.0	40.9	960	27.8	17.2	703	29.7	28.0	1,039	29.2
	B	32.9	1,366	28.0	32.8	1,517	31.6	63.1	2,200	29.1	22.0	953	30.6	37.7	1,509	29.8
	C	33.5	1,659	27.4	36.9	1,385	31.4	54.2	1,680	27.8	24.8	1,018	29.8	37.4	1,436	29.1
	D	39.6	1,776	27.4	46.4	2,248	31.8	77.5	1,900	25.7	25.7	1,301	29.7	47.3	1,806	28.7
Franklin	A	32.9	1,600	28.5	33.3	1,035	32.7	45.2	1,200	26.2	19.0	926	29.1	32.6	1,190	29.1
	B	40.9	1,815	29.1	39.6	1,844	32.3	72.5	2,660	28.2	25.4	1,116	30.5	44.6	1,859	30.0
	C	42.1	2,166	28.8	43.3	1,649	33.8	73.8	3,320	26.7	26.2	1,230	29.8	46.4	2,091	29.8
	D	46.4	2,186	28.4	53.0	2,271	33.1	79.1	3,700	25.9	25.1	1,548	29.5	50.9	2,426	29.2
Gopher	A	37.8	1,522	27.6	28.9	1,175	33.3	53.5	1,000	26.2	17.3	661	30.0	34.4	1,090	29.3
	B	38.4	1,347	27.7	36.0	1,727	33.1	78.1	2,740	25.8	23.1	838	30.6	43.9	1,663	29.3
	C	43.3	1,737	27.2	38.9	1,478	34.1	68.2	2,880	23.9	24.4	994	30.5	43.7	1,772	28.9
	D	48.8	1,952	27.0	49.8	2,061	34.6	51.2	2,800	24.0	28.3	1,211	30.3	44.5	2,006	29.0
Big Four	A	36.6	2,030	27.6	32.1	1,073	32.2	41.6	1,160	26.3	21.4	841	28.5	32.9	1,276	28.7
	B	42.1	1,776	27.5	41.6	1,937	32.3	67.8	2,600	26.7	24.7	990	28.8	44.1	1,826	28.8
	C	45.1	1,991	27.2	45.2	1,898	33.2	68.9	3,260	26.1	25.9	1,271	30.0	46.3	2,105	29.1
	D	51.2	2,186	27.0	53.2	2,341	33.4	63.7	4,120	24.9	28.1	1,450	30.3	49.1	2,524	28.9
Wayne	A	39.0	1,717	26.9	30.6	980	33.0	46.1	1,640	27.1	16.0	734	27.6	32.9	1,268	28.7
	B	40.9	1,581	27.0	41.6	1,330	32.9	74.5	2,680	28.2	23.0	1,006	28.6	45.0	1,649	29.2
	C	43.9	1,952	26.9	44.8	1,435	33.5	80.8	3,340	28.0	25.9	1,208	28.3	48.9	1,984	29.2
	D	50.0	2,225	26.8	53.2	1,704	33.6	65.2	4,700	25.7	26.3	1,343	29.7	48.7	2,493	29.0
Markton	A	32.3	1,542	25.4	39.1	1,470	32.6	47.4	1,220	26.1	18.7	716	28.3	34.4	1,237	28.1
	B	27.4	1,308	24.2	44.0	2,014	32.8	73.8	2,160	27.6	24.7	906	29.3	42.5	1,597	28.5
	C	31.7	1,639	25.1	51.5	1,696	33.6	74.6	2,760	26.2	24.7	1,187	30.5	45.6	1,821	28.9
	D	37.2	1,932	24.1	63.0	2,730	33.6	76.8	3,460	24.7	26.6	1,297	31.8	50.9	2,355	28.6
Miami	A	45.1	1,835	29.2	33.8	1,175	31.7	46.5	1,160	26.0	19.7	800	28.0	36.3	1,243	28.7
	B	36.6	1,561	27.9	39.4	2,085	31.7	71.2	2,480	26.4	26.1	1,040	28.6	43.3	1,792	28.7
	C	45.7	2,205	27.9	40.6	1,346	32.8	70.2	2,740	26.2	26.6	1,413	29.1	45.8	1,926	29.0
	D	54.9	2,537	28.3	51.0	2,723	32.1	71.9	3,940	24.2	26.9	1,457	28.4	51.2	2,664	28.3

TABLE 2.—*Yield per acre and weight per bushel of 17 varieties of oats grown at 4 levels of soil fertility, 1929–32*—Continued

Variety	Level	1929			1930			1931			1932			Average		
		Grain	Straw	Weight per bushel	Grain	Straw	Weight per bushel	Grain	Straw	Weight per bushel	Grain	Straw	Weight per bushel	Grain	Straw	Weight per bushel
		Bushels	*Pounds*	*Pounds*	*Bushels*	*Pounds*	*Pounds*	*Bushels*	*Pounds*	*Pounds*	*Bushels*	*Pounds*	*Pounds*	*Bushels*	*Pounds*	*Pounds*
Cornellian	A	47.0	2,088	29.3	36.0	1,338	32.8	48.1	1,020	26.9	25.7	898	29.6	39.2	1,336	29.7
	B	30.6	1,854	28.7	43.8	2,100	32.8	72.0	2,620	27.7	27.8	1,052	30.0	45.8	1,907	29.8
	C	49.4	2,244	28.6	50.1	1,742	33.0	68.7	3,340	26.9	30.0	1,360	30.6	49.6	2,172	29.8
	D	57.9	2,596	28.5	56.9	2,691	34.1	68.3	2,580	25.1	30.0	1,608	30.0	53.3	2,369	29.4
Silvermine	A	36.0	1,659	26.3	28.2	1,354	32.0	42.8	1,440	26.2	19.6	781	29.1	31.7	1,309	28.4
	B	36.0	1,425	26.1	36.7	1,626	32.0	69.0	3,320	27.3	25.6	1,140	29.1	41.8	1,878	28.6
	C	38.4	1,659	26.7	42.3	2,069	33.3	66.7	3,180	25.1	27.9	1,310	30.0	43.8	2,055	28.8
	D	45.1	2,147	26.3	46.4	2,248	33.0	71.1	3,340	25.5	26.3	1,447	30.5	47.2	2,296	28.8
Victory	A	42.1	1,776	26.9	36.5	1,322	31.5	45.3	1,200	25.6	36.5	883	28.0	40.1	1,295	28.0
	B	38.4	1,815	25.0	47.6	1,898	31.7	68.6	2,480	26.4	47.6	1,318	27.9	50.6	1,878	27.8
	C	37.8	2,147	26.4	53.2	2,264	31.9	77.7	3,260	26.7	53.2	1,549	28.5	55.5	2,305	28.4
	D	54.9	2,459	27.3	55.4	2,349	33.1	73.1	3,440	25.1	55.4	1,761	28.3	59.7	2,502	28.5
Lincoln	A	33.5	1,581	22.4	30.4	1,206	28.2	42.4	1,080	24.4	19.0	799	25.7	31.3	1,167	25.2
	B	29.3	1,483	22.3	40.6	1,657	27.7	66.4	2,620	25.1	24.9	1,109	26.2	40.3	1,717	25.3
	C	33.5	1,737	22.5	41.1	1,952	28.9	65.1	3,060	24.7	26.7	1,239	27.1	41.6	1,997	25.8
	D	42.7	2,225	21.8	48.6	2,015	28.9	66.9	2,920	23.2	23.6	1,500	27.6	45.5	2,165	25.4
Wolverine	A	35.4	1,444	27.6	31.6	1,400	33.5	48.2	940	24.3	21.6	809	27.4	34.2	1,148	28.2
	B	31.7	1,327	27.5	43.5	1,952	33.4	71.9	2,760	24.4	24.1	1,011	27.9	42.8	1,763	28.3
	C	39.0	1,639	27.2	45.5	1,890	34.5	74.6	3,300	25.2	27.0	1,337	28.2	46.5	2,042	28.8
	D	46.4	2,030	27.9	57.4	2,520	35.2	71.7	3,020	22.8	26.0	1,438	28.8	50.4	2,252	28.7
Ohio 201	A	37.2	2,166	25.5	39.4	1,618	29.7	45.1	1,360	23.7	24.3	964	28.1	36.5	1,527	26.8
	B	36.0	1,893	25.4	49.3	2,777	29.7	72.7	3,360	25.3	28.7	1,423	28.9	46.7	2,363	27.3
	C	41.5	2,654	25.7	53.5	2,722	30.4	74.4	4,100	25.3	31.1	1,604	29.1	50.1	2,770	27.6
	D	51.8	3,181	26.0	58.1	3,275	31.2	75.6	3,980	22.5	32.5	1,838	30.8	54.5	3,069	27.6
Ohio 19607	A	39.0	2,108	25.8	40.1	1,750	29.8	45.4	1,420	24.6	22.9	979	27.0	36.9	1,564	26.8
	B	34.8	1,776	26.2	45.0	2,528	29.5	74.8	3,220	25.2	27.1	1,413	26.0	45.4	2,234	26.7
	C	40.3	2,303	25.7	53.2	2,652	30.5	84.7	4,300	25.7	27.9	1,767	28.5	51.5	2,756	27.6
	D	51.2	3,123	27.1	60.0	2,979	31.0	70.4	3,760	23.4	28.9	1,688	29.0	52.6	2,888	27.6
Average	A	36.4	1,667	26.9	31.6	1,195	31.5	44.9	1,174	25.8	21.3	810	28.2	33.6	1,212	28.1
	B	35.4	1,522	26.6	39.8	1,783	31.6	70.0	2,608	26.7	26.5	1,059	28.8	42.9	1,743	28.4
	C	39.8	1,909	26.6	44.1	1,767	32.3	70.6	3,102	25.9	28.5	1,285	29.1	45.8	2,016	28.5
	D	47.5	2,235	26.7	52.1	2,278	32.6	69.2	3,348	24.5	29.0	1,456	29.5	49.5	2,329	28.3

Plots were cut with an ordinary grain binder, and uniform stubble was left on all levels. For this reason straw yields are relatively too low on the A level, where there was definitely less vegetative growth. The errors introduced by the unharvested stubble at the higher levels are of little importance.

A study of figure 1 shows that there were marked differences between seasons. In 1931 the yields of both grain and straw were high; in 1932 they were decidedly low. The influence of season must, therefore, be considered in interpreting the experiment. Table 3 gives meteorological data at Wooster for all four seasons.

TABLE 3.—*Mean maximum and minimum temperatures and precipitation for Wooster, Ohio, during the growing season for oats, 1929–32*

Month	Mean maximum temperature				Mean minimum temperature				Precipitation			
	1929	1930	1931	1932	1929	1930	1931	1932	1929	1930	1931	1932
	°F.	°F.	°F.	°F.	°F.	°F.	°F.	°F.	*Inches*	*Inches*	*Inches*	*Inches*
April	63.0	64.0	61.6	60.0	40.4	38.0	35.2	33.0	5.58	2.23	4.10	2.55
May	70.6	74.3	70.2	72.2	44.8	48.0	44.6	45.4	4.84	1.59	4.45	1.93
June	79.0	84.1	81.2	82.6	53.0	53.9	54.3	55.2	4.10	2.86	3.49	3.44
July	83.9	90.4	89.3	84.8	59.3	57.4	61.9	59.2	6.79	1.71	2.97	3.14
August	80.3	86.0	83.7	85.6	52.4	55.1	59.9	56.0	1.26	2.64	4.68	2.01

In 1929 there was a wet, relatively warm spring, followed by a dry period during the latter part of May and early June. Summer temperatures were not excessive, and rainfall was plentiful after mid-June.

The season of 1930 was somewhat cooler in early spring, and warm in summer. Precipitation was lower in April and early May. Through the rest of May and in June and July the weather was very dry.

In 1931 conditions were more favorable than in other years. Distribution of rainfall was very satisfactory, and in spite of high temperatures in late June and July oats yielded well. Test weight was low, however.

The temperature in the spring of 1932 was not satisfactory, and precipitation was low in May and early June. Oats made poor vegetative growth and gave low yields of grain.

ANALYSIS OF VARIANCE

Analysis of variance by the method developed by Fisher [4] offered a satisfactory means of handling the data. To estimate odds for significance Snedecor's F [5] was used.

Table 4 presents data from the analyses of total yield (grain+straw) and of grain yield for each season. Variety, level, block, and the variety-level interaction were considered. Because of the smaller area of land in one of the fields, three varieties were sown in only two blocks in 1930. Calculations were made on the actual data, and again with estimated yields introduced for the 12 missing plots. The theoretical third replication was given the mean yield of the two actually grown. Such a procedure would be expected to give too low

[4] FISHER, R. A. STATISTICAL METHODS FOR RESEARCH WORKERS. Ed. 4, 302 pp., illus. Edinburgh and London. 1932.

[5] SNEDECOR, G. W. CALCULATION AND INTERPRETATION OF VARIANCE AND COVARIANCE. 96 pp. Ames, Iowa. 1934. (Iowa State Col., Div. Indus. Sci. Monog. 1.)

an estimate of variance, but in this case it changed the F values very little, and the significance not at all. Any error introduced, therefore, had no practical importance, and the estimated plot yields were used because they permitted the analysis of data for all seasons together with greater facility.

TABLE 4.—*Analysis of variance, by seasons, of total yield and of grain yield for 17 varieties of oats grown at 4 fertility levels in 4 seasons*

TOTAL YIELD

Season	Variance due to—	Degrees of freedom	Sums of squares	Variance	F	1 percent point for F [1]	5 percent point for F [1]	
1929	Variety	16	642.54	40.159	15.25	2.20	1.75	
	Level	3	657.59	219.197	83.22	3.94	2.68	$\bar{x}$=13.26 pounds.
	Block	2	102.39	51.195	19.44	4.78	3.07	σ=1.62 pounds.
	Variety-level interaction	48	88.25	1.839	.70	1.80	1.50	C. V.=12.22 percent.
	Error	134	352.94	2.634				
	Total	203	1,843.71					
1930	Variety	16	915.18	57.199	14.22	2.20	1.75	
	Level	3	1,594.80	531.600	132.17	3.94	2.68	$\bar{x}$=13.54 pounds.
	Block	2	43.76	21.880	5.44	4.78	3.07	σ=2.01 pounds.
	Variety-level interaction	48	121.95	2.541	.63	1.80	1.50	C. V.=14.84 percent.
	Error	134	538.91	4.022				
	Total	203	3,214.60					
1931	Variety	16	822.74	51.421	5.29	2.20	1.75	
	Level	3	5,153.23	1,717.743	176.56	3.94	2.68	$\bar{x}$=19.66 pounds.
	Block	2	32.32	16.160	1.66	4.78	3.07	σ=3.12 pounds.
	Variety-level interaction.	48	579.69	12.077	1.24	1.80	1.50	C. V.=15.87 percent.
	Error	134	1,303.68	9.729				
	Total	203	7,891.66					
1932	Variety	16	134.92	8.433	11.91	2.20	1.75	
	Level	3	399.95	133.317	188.30	3.94	2.68	$\bar{x}$=8.42 pounds.
	Block	2	54.43	27.215	38.44	4.78	3.07	σ=.84 pounds.
	Variety-level interaction.	48	21.55	.449	.63	1.80	1.50	C. V.=9.98 percent.
	Error	134	94.90	.708				
	Total	203	705.75					

GRAIN YIELD

1929	Variety	16	3,861.01	241.313	11.91	2.20	1.75	
	Level	3	4,659.80	1,553.267	76.69	3.94	2.68	$\bar{x}$=39.55 bushels.
	Block	2	1,403.19	701.595	34.64	4.78	3.07	σ=4.50 bushels.
	Variety-level interaction.	48	834.37	17.383	.86	1.80	1.50	C. V.=11.38 percent.
	Error	134	2,714.14	20.255				
	Total	203	13,472.51					
1930	Variety	16	6,451.46	403.216	18.89	2.20	1.75	
	Level	3	11,225.41	3,741.803	175.30	3.94	2.68	$\bar{x}$=41.94 bushels.
	Block	2	325.05	162.525	7.61	4.78	3.07	σ=4.62 bushels.
	Variety-level interaction.	48	439.09	9.148	.43	1.80	1.50	C. V.=11.02 percent.
	Error	134	2,860.28	21.345				
	Total	203	21,301.29					
1931	Variety	16	2,466.25	154.141	2.71	2.20	1.75	
	Level	3	23,822.65	7,940.883	139.37	3.94	2.68	$\bar{x}$=63.48 bushels.
	Block	2	266.39	133.195	2.34	4.78	3.07	σ=7.55 bushels.
	Variety-level interaction.	48	3,870.02	80.625	1.42	1.80	1.50	C. V.=11.89 percent.
	Error	134	7,634.61	56.975				
	Total	203	38,059.92					
1932	Variety	16	647.08	40.443	9.92	2.20	1.75	
	Level	3	1,495.39	498.463	122.29	3.94	2.68	$\bar{x}$=24.91 bushels.
	Block	2	591.88	295.940	72.61	4.78	3.07	σ=2.02 bushels.
	Variety-level interaction.	48	263.94	5.499	1.35	1.80	1.50	C. V.=8.11 percent.
	Error	134	546.12	4.076				
	Total	203	3,544.41					

[1] Approximate values.

Total yield represents the total top growth, independent of how much of the material moves into the developing kernels. Grain yield, on the other hand, measures particularly the translocation of elaborated plant food into the developing embryo and endosperm. A general comparison of the two parts of table 4 indicates that after variance is removed for the factors considered, residual error is greater for total than for grain yields. This is measured by the coefficient of variability (C. V.), since the standard deviation (σ) has been estimated from the variance for error. This is surprising, since the filling of the grain follows the virtual completion of vegetative growth, and grain yields, therefore, would be expected to show greater variability because of the additional physiological processes involved.

A second unexpected reaction appears in comparing the 1931 results with those of other years. This was the season when oats gave their highest yields in both straw and grain, but the coefficients of variability for both total yields and grain yields were the largest obtained because of the large variance for error. Conversely, the yields and coefficients were both lowest in 1932. This implies that factors introducing variability other than variety, level, and the interaction of these two, become increasingly important as the season is more favorable, and the yields are higher.

Differences among varieties were highly significant in all cases, but the F values were decidedly lower in 1931. This does not necessarily indicate that the spread among varieties was greater with the more adverse seasonal conditions. The greater unaccounted-for error in 1931 is largely responsible for the lower significance in that year.

Fertility was a very important factor because of the wide range between the A level and the D level. The spread among the different treatments, however, varied with season as was to be expected. Leaching, precipitation, and temperature probably all played important roles, especially since the oats crop received no direct applications of fertilizer.

A comparison of the variance for variety with the varietal means, for grain and for total yields, may be made by considering the ratio $\frac{\sqrt{\text{Variance due to variety}}}{\text{Mean varietal yield}}$. In all four seasons this ratio is greater for total than for grain yields. In 1929 it is 1.22 times; in 1930, 1.17 times; in 1931, 1.87; and in 1932, 1.33 times as great.

For optimum development oats require a cool, moist season. The Ohio climate is by no means ideal for them. It may be that the relatively cool weather before heading is favorable enough that the inherent differences between varieties can find expression in the vegetative growth, whereas the hot weather following heading is so unfavorable that varietal response in the grain yields is inhibited in some degree.

The variety-level interaction is one of the most important points studied. In no single season did it prove mathematically significant, though in 1931 the F value approaches the 5-percent point closely, especially for the grain yields. There is a more nearly significant interaction for grain than for total yields in 3 of the 4 years, indicating a possible differential response in the reproductive phases greater than in purely vegetative growth. The evidence for this, however, is far from conclusive.

Definite indication of differences between blocks was found, especially on that field used in 1929 and 1932. In fact, in most cases the variety-block interaction was significant and, strangely enough, in all instances except for grain in 1931, this interaction was more significant than that of variety-level. This represents a serious error in the experiment, which, however, cannot be justifiably removed, since it represents an uncontrolled and largely indeterminate factor. It is probably much less important as a source of error in the analyses presented in table 5, where all seasons are taken together. Since three fields were used, variance for block could not be removed directly, and the total yield of the three replicates was used as the starting point for this anaysis.

TABLE 5.—*Analysis of variance for total yield and grain yield of 17 varieties of oats grown at 4 levels of soil fertility during 4 seasons*

TOTAL YIELD

Variance due to—	Degrees of freedom	Sums of squares	Variance	F	1-percent point for F [1]	5-percent point for F [1]	
Variety	16	5,611.10	350.694	29.00	2.12	1.70	
Level	3	16,263.13	5,421.043	448.28	3.91	2.66	
Season	3	38,933.07	12,977.690	1,073.16	3.91	2.66	$\bar{x}$=13.72 pounds.
Variety-level interaction	48	692.99	14.437	1.19	1.75	1.40	σ=1.16 pounds.
Variety-season interaction.	48	1,935.05	40.314	3.33	1.75	1.40	C. V.=8.45 percent.
Level-season interaction.	9	7,153.55	794.839	65.73	2.63	2.00	
Error	144	1,741.33	12.093				
Total	271	72,330.22					

GRAIN YIELD

Variety	16	21,967.39	1,372,962	14.64	2.12	1.70	
Level	3	81,874.57	27,291.523	291.07	3.91	2.66	
Season	3	464,226.48	154,742.160	1,650.35	3.91	2.66	$\bar{x}$=42.47 bushels.
Variety-level interaction	48	2,720.43	56.676	.60	1.75	1.40	σ=3.23 bushels.
Variety-season interaction.	48	18,310.02	381.459	4.07	1.75	1.40	C. V.=7.60 percent.
Level-season interaction.	9	41,735.19	4,637.243	49.46	2.63	2.00	
Error	144	13,501.81	93,763				
Total	271	644,335.89					

[1] Approximate values.

Without question varieties differed in yielding ability, and the variance due to levels was large, but neither of these factors exerted as much influence as season. The variety-level interaction was not mathematically significant in any case. However, when variety-block and level-block variance was removed from error, this interaction reached the level of significance in one season in the case of total yields, and in three seasons in the case of grain yields. Nevertheless when all seasons were considered together, no significant variety-level interaction was found. This was in marked contrast to the wheat crop in the rotation, where, in the corresponding analysis, odds exceeded 99:1 for total yields, and approached the 1-percent point for grain yields.

Consideration of the data from all analyses indicates that for oats the variety-level interaction is of questionable significance mathematically, and the actual yield differences involved are of no practical

significance. It therefore appears safe to apply variety-test results obtained at Wooster to rather divergent soil-fertility conditions, since the unaccounted-for errors are relatively low.

SUMMARY AND CONCLUSIONS

A 3-year rotation of corn, oats, and winter wheat has been grown at four fertility levels at the Ohio Agricultural Experiment Station. Oats alone are considered in this paper.

Data on grain yield, straw yield, and weight per bushel are given for 17 varieties of oats, all grown for four seasons.

Climatological data covering the period of the experiment are included.

Analysis of variance for total yield and for grain yield, by individual seasons, and all seasons together, indicates that differences due to variety, to level, and to season are all highly significant. The variety-season interaction is highly significant, but the variety-level interaction is probably not significant.

Unaccounted-for error, as measured by the coefficient of variability, is less for grain than for total yield. This is unexpected, and may be due to the wide divergence of climate from the ideal for the crop.

Because the variety-level interaction is of questionable significance, and at the same time the unaccounted-for errors are low, it appears safe to apply results of a variety oat experiment at Wooster, Ohio, to a rather wide range of soil conditions.

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SOME EFFECTS OF STORAGE CONDITIONS ON CERTAIN DISEASES OF LEMONS [1]

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INTRODUCTION

The presence of carbon dioxide in a lemon-storage room is quite generally regarded as harmful. Many rooms are operated under instructions that the carbon dioxide in the atmosphere must be kept below 0.5 percent.

Low temperature is known to be harmful to lemons, and exposure to temperatures below 50° F., even during a shipping period of 1 or 2 weeks, is usually carefully avoided.

The present study was undertaken to obtain more definite information in regard to the carbon dioxide and low-temperature tolerance of lemons (*Citrus limonia* Osbeck) and the nature of the injuries resulting from unfavorable storage conditions.

In the course of the experiments several different diseases developed, and in most cases the frequency of their occurrence was modified by the storage treatment.

MATERIALS AND METHODS

The lemons used in the experiments were forwarded under ventilation in carlot shipments from Los Angeles, Calif.,[2] to New York City and were then shipped by ordinary express to Washington, D. C. They were hauled to the Arlington Experiment Farm, Arlington, Va., and the experiments were started there within a day from the time of arrival in Washington.

The term "green lemons" is used for fruit that at the time of shipment was dark green in color with only very slight traces of yellow, the term "silver lemons" for fruit that was turning from green to yellow, and the term "mature lemons" for fruit that was bright yellow and of proper maturity for immediate marketing. At the time the experimental treatments were begun, the green and silver lemons had slightly too much yellow to be typical for the terms applied.

In the earlier experiments, 20 to 30 lemons of a particular maturity were used under each storage condition. In the later experiments, this number was increased to 50 or 60 lemons for most of the tests. The lemons were selected individually, and great care was taken that the different lots should be alike in maturity, firmness, and general appearance.

It was intended that the relative humidity of the storage rooms should be maintained at about 88 percent; but it sometimes fell

[1] Received for publication Dec. 30, 1936; issued February 1937.

[2] The writers are indebted to C. W. Mann, of the Division of Fruit and Vegetable Crops and Diseases, for the selection and shipment of the fruit.

several points below this for short periods, especially in the 60° F. room. All records of temperature are reported in degrees Fahrenheit.

In part of the carbon dioxide experiments the lemons were held in 5-gallon jars during the period of treatment. In some cases the desired atmosphere was maintained by continuous renewal with air to which carbon dioxide had been added by means of flowmeters. In other cases the carbon dioxide was built up by means of the respiration of the lemons. In the latter tests a fair degree of uniformity was secured by occasional analyses and the regulation of a small opening in the container. In other tests a high percentage of carbon dioxide was maintained for short periods by the use of solid carbon dioxide as a supplement to ordinary ice in pony refrigerators. In these experiments both the carbon dioxide and the temperature were lower at the end of the treatment than at the beginning.

The waxes used in the experiments were prepared by mixing paraffin and mineral oil in approximately equal proportions. In some cases beeswax was substituted for part of the paraffin. There was no indication that the results were affected by modifications in the formula for the wax.

DECAY

CHARACTERISTICS

The most common form of decay was that caused by *Alternaria citri* Pierce. Much of this decay was of the "center" type, the central axis or core of the lemon becoming black and soft, with little external evidence of the disease, as is shown in figure 1, *A*. In other cases the entire lemon became black and soft, the decay usually starting at the button and moving toward the stylar end (fig. 1, *B*).

Penicillium digitatum Sacc. was a close second to *Alternaria* as a cause of decay, and *P. italicum* Wehmer was of somewhat less common occurrence. The type of decay produced by the last-named is shown in figure 1, *C*.

EFFECT OF TEMPERATURE

During the first 6 to 8 weeks of storage there was greater development of decay at the higher than at the lower temperatures. This was especially true of alternaria decay. When the storage period was prolonged to 10 weeks or more there was sometimes more decay at 32° and 36° F. than at any of the higher temperatures, especially with fruit that had been stored at the green or the silver stage of maturity. This greater decay at the lower temperatures was largely due to one or the other of the *Penicillium* species and was apparently the result of a prior development of pitting, watery break-down, or other low-temperature trouble that gave the decay organisms favorable points of entrance.

In lemons that were held 1 or 2 weeks at 32°, 36°, or 40° F. before being stored at 50° or 60° there was in general but slightly less decay than in the fruit held continuously at the higher temperature, especially after long periods of storage.

EFFECT OF CARBON DIOXIDE AND WAXING

It was pointed out in an earlier publication [3] that carbon dioxide has a decidedly inhibiting effect on penicillium decay of oranges and

[3] BROOKS, C., BRATLEY, C. O., and MCCOLLOCH, L. P. TRANSIT AND STORAGE DISEASES OF FRUITS AND VEGETABLES AS AFFECTED BY INITIAL CARBON DIOXIDE TREATMENTS. U. S. Dept. Agr. Tech. Bull. 519, 24 pp., illus. 1936.

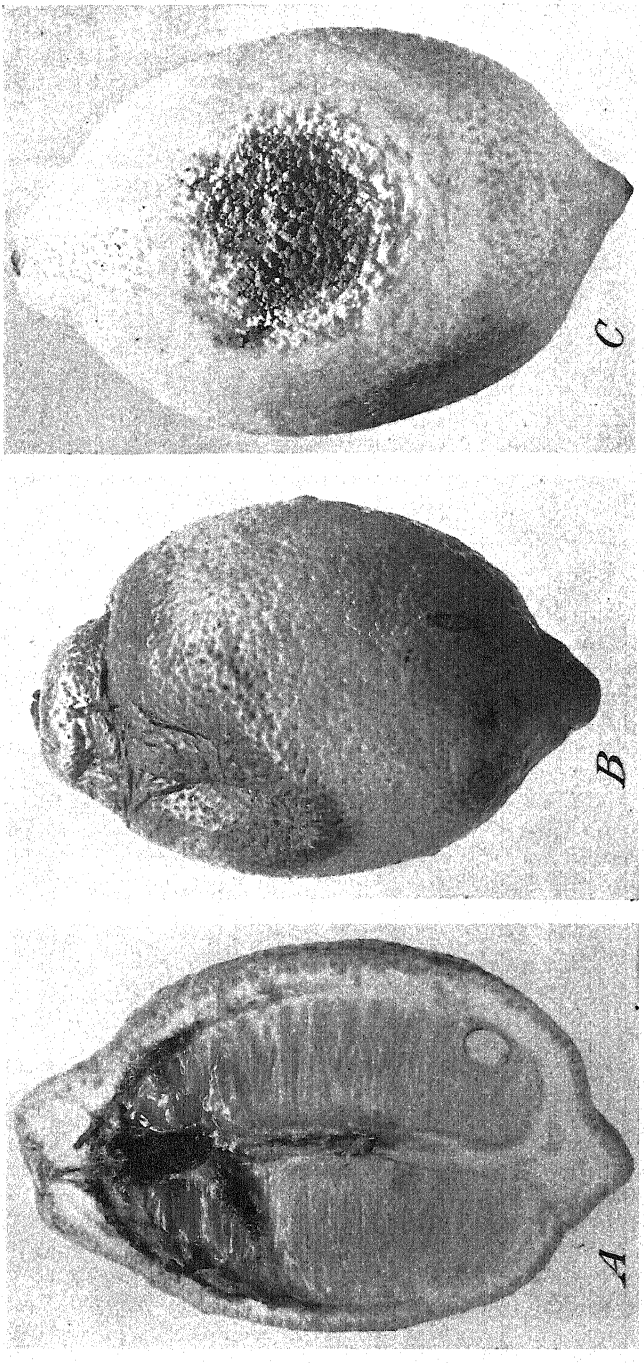


FIGURE 1.—Decay of lemons caused by *Alternaria citri* (*A* and *B*) and by *Penicillium italicum* (*C*). Note the spread along the central axis in *A* and the more lightly colored zone around the central area in *C*.

grapefruit. In the present experiments the effect of carbon dioxide was tested on lemons that had been inoculated with *Alternaria citri*. The development of this decay was slow under all conditions, but the progress was apparently as great in an atmosphere containing 40 percent of carbon dioxide as in one that was practically free from carbon dioxide.

A record of decay was kept in all the carbon dioxide and waxing experiments that are reported in detail under pitting and membranous stain. The results gave no indication that either waxing or continuous exposure to low percentages of carbon dioxide (10 percent or less) had any significant effect upon the percentage of either penicillium or alternaria decay at the end of long storage periods.

Lemons that were held in tight containers for long-period carbon dioxide treatments or as controls for these treatments developed more decay in some instances than the fruit that was held in commercial packages at the same temperature. This difference was apparently due to a higher humidity in the tight containers. Fawcett, Klotz, and Nixon [4] have reported experiments in which alternaria decay was reduced by air conditioning.

PITTING AND PETECA

CHARACTERISTICS

Pitting, as the name implies, is characterized by the development of pits or depressions in the rind of the fruit,[5] as shown in figure 2, *A* and *B*. In the present experiments it took various forms, its characteristics as well as its prevalence varying with the treatment and apparently with the maturity and vitality of the fruit. In many cases the color of the rind in the pitted area remained normal or nearly so; in other cases it became light brown or a dark brown approaching black.

The pits were usually as much as 0.2 inch in diameter and often more than 0.5 inch, but under certain storage conditions a smaller and shallower type prevailed, giving the lemons a somewhat speckled appearance, as shown in figure 2, *C*. This type was particularly common in 36° storage.

Peteca resembles pitting, but the surface of the peel is relatively slow in losing its color and in the early stages the oil glands are darker than the surrounding tissue.[5] This is shown in figure 2, *D*.

EFFECT OF TEMPERATURE

The effect of temperature upon the development of pitting and peteca is shown in figures 3 to 9, inclusive. Pitting did not occur in storage at 60° F. It was usually absent and never of importance with either green or mature lemons held at 50°, but in a few instances the silver lemons developed the disease in 50° storage. At 32°, 36°, and 40° the lemons were often badly pitted, especially when held in storage more than 8 to 10 weeks. After the lemons had been 8 weeks or more in storage the disease was much worse at 32° and 36° than at 40°, and with green lemons it was usually worse at 36° than at 32°. These contrasting temperature effects did not disappear when the

[4] FAWCETT, H. S., KLOTZ, L. J., and NIXON, H. W. EFFECTS OF STORAGE AND HOLDING CONDITIONS ON ALTERNARIA IN LEMONS. Calif. Citrogr. 21: 118, 143-144. 1936.

[5] FAWCETT, H. S., and LEE, H. A. CITRUS DISEASES AND THEIR CONTROL. New York. 1926. [Also ed 2, by Fawcett. 1936.]

fruit was held at room temperature for a week after removal from storage; in fact, they were usually somewhat emphasized by such holding. The pits developed at 40° were often darker than those at 32°. This may have been due to greater aging, greater oxidation, or perhaps to some other cause.

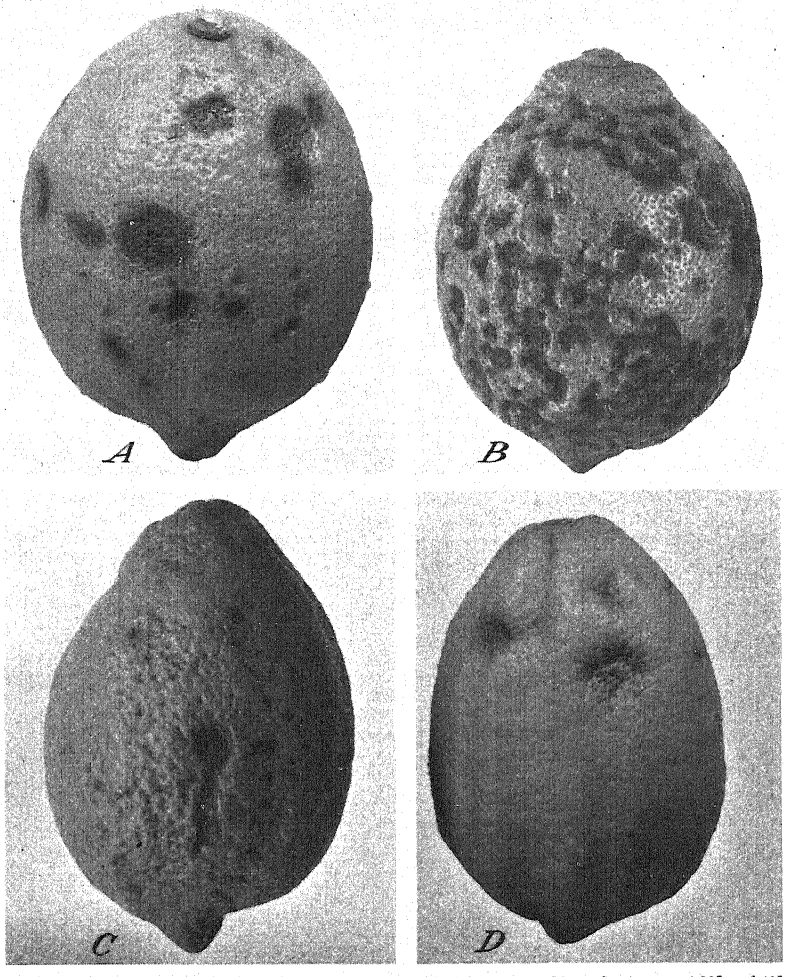


FIGURE 2.—Pitting and peteca: *A*, Lemon showing deep pitting, the usual type in storage at 32° and 40° F.; *B*, pitting on a green lemon that was held at 36° for 13 weeks; *C*, lemon showing shallow pitting, the most common type in storage at 36°, and also one deep pit; *D*, peteca on lemon, as found after shipment from California to Washington, D. C.

Green, silver, and mature lemons that were held at 32°, 36°, or 40° F. for 1 or 2 weeks and were then stored at 60° remained free from pitting. Lemons similarly held at 32° and stored at 50° remained as free from pitting on the average as those held continuously at 50°. Lemons similarly held at 36° or 40° before being stored at 50° showed a slight increase in pitting in some instances, as compared with those held continuously at 50°. There was little to indicate that holding

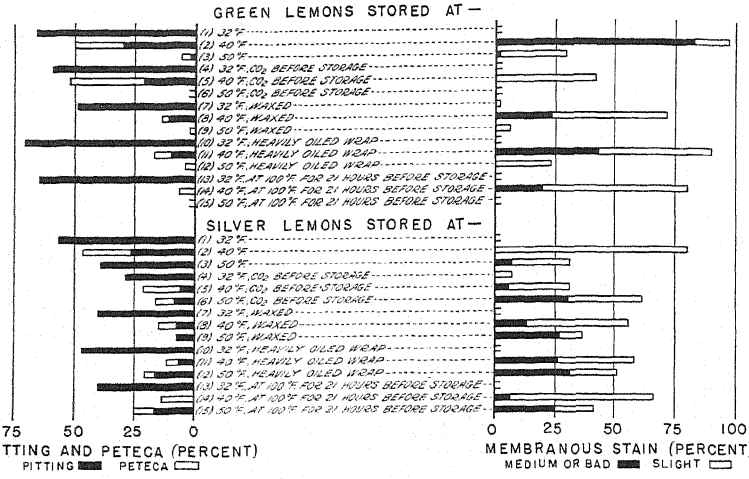


FIGURE 3.—Effect of storage conditions on pitting, peteca, and membranous stain. Green and silver lemons stored at various temperatures November 28, 1933; record made 14 weeks later. In the lots receiving CO_2 before storage, the fruit was held for 64 hours in an atmosphere in which the carbon dioxide dropped from 50 percent to 30 percent and averaged 36 percent and in which the temperature dropped from 70° to 50° F. and averaged 56°.

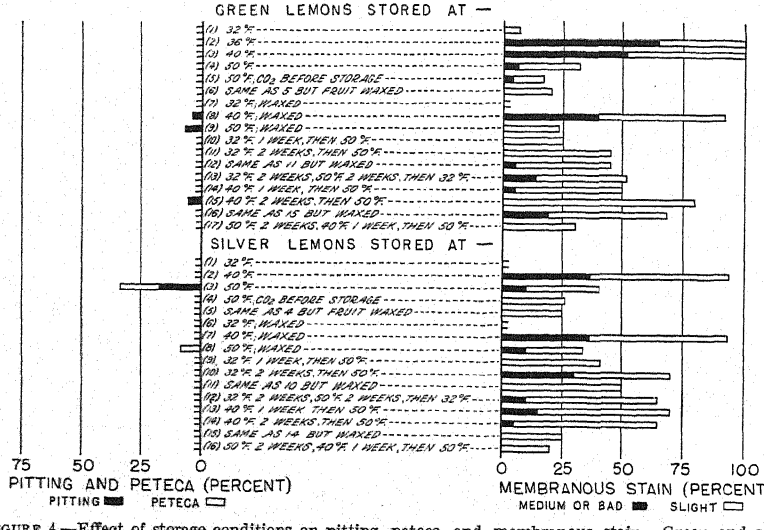


FIGURE 4.—Effect of storage conditions on pitting, peteca, and membranous stain. Green and silver lemons stored at various temperatures March 20, 1934; record made 12 weeks later. In the lots receiving CO_2 before storage the fruit was held for 48 hours at 59° F. in an atmosphere in which the carbon dioxide was maintained at 50 percent by continuous air renewal.

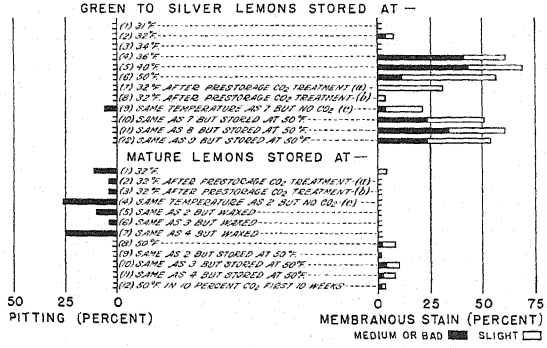


FIGURE 5.—Effect of storage conditions on pitting and membranous stain (no peteca developed) of lemons stored at various temperatures June 23, 1934. Part of the lemons were about halfway between the green and silver stages in maturity and the others were fully mature; record made after 12 weeks' storage: *a*, Lemons in a pony refrigerator with the temperature dropping from 73° to 49° F. in 42 hours and averaging 51°, and the carbon dioxide dropping from 59 percent to 30 percent and averaging 40 percent; *b*, temperature as above, but the carbon dioxide dropping from 33 percent to 14 percent and averaging 22 percent; *c*, controls, with no carbon dioxide.

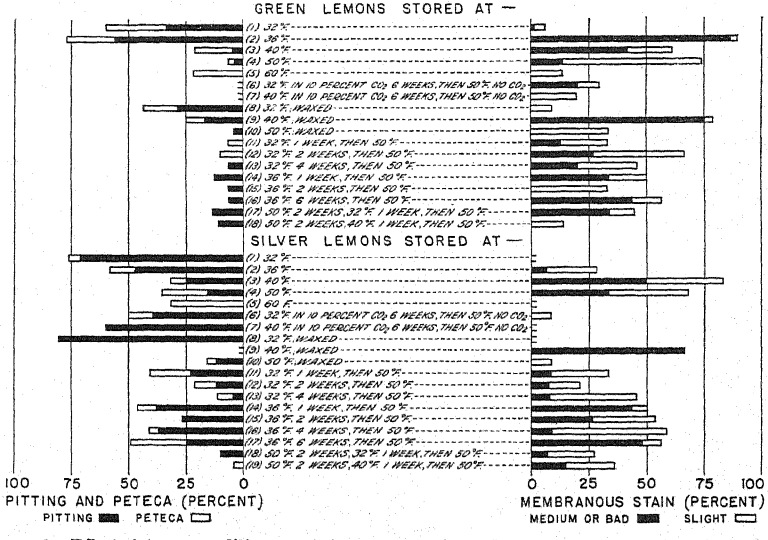


FIGURE 6.—Effect of storage conditions on pitting, peteca, and membranous stain. Green and silver lemons stored at various temperatures January 23, 1935; record made 16 weeks later. In the lots exposed to CO_2 the gas was built up by the respiration of the lemons and the treatments were continued during the first 6 weeks of storage.

the fruit at a lower temperature for 1 or 2 weeks, or even 4 weeks, produced any decided tendency to pitting.

In the experiments reported in figure 3, fruit that was held at 100° F. for 21 hours before being stored had slightly less pitting than the control fruit, but the difference could hardly be regarded as significant.

There seemed to be little evidence that the development of peteca was modified by temperature conditions. Its erratic occurrence with some lots of fruit seemed to indicate that its prevalence was largely determined by prestorage conditions.

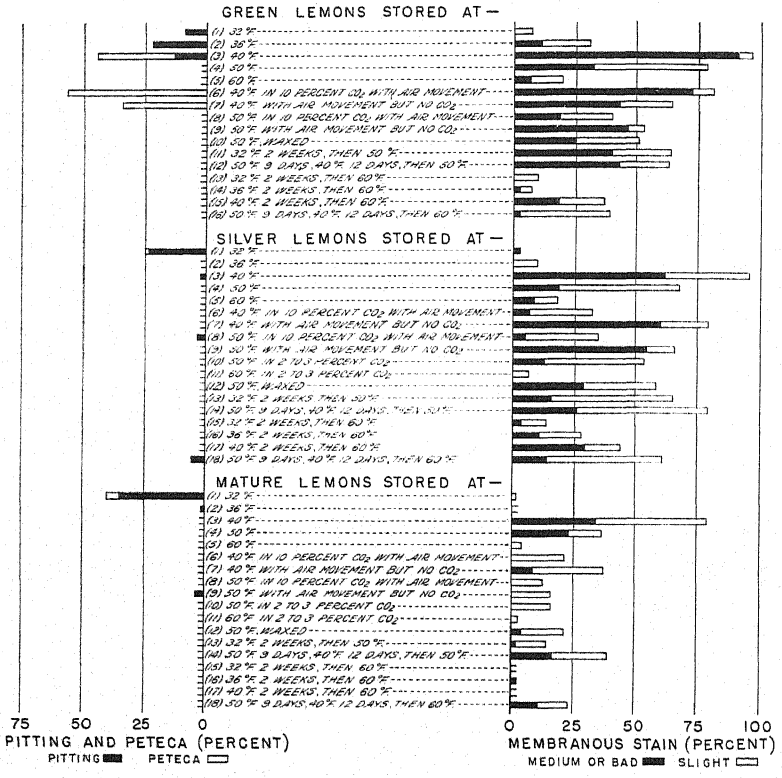


FIGURE 7.—Effect of storage conditions on pitting, peteca, and membranous stain of lemons stored at various temperatures June 26, 1935. The carbon dioxide treatments were continued during the first 3 weeks of storage. The record on the silver and mature lemons was after 9 weeks' storage, that on the green lemons after 14 weeks' storage.

EFFECT OF CARBON DIOXIDE

In several of the experiments lemons were exposed to high percentages of carbon dioxide for a period of 2 or 3 days before being placed in storage. The results are shown in figures 3 to 5, inclusive. In four tests with green lemons, four with silver lemons, and two with mature lemons, pitting was decreased by this prestorage gas treatment, and there was no instance in which it was increased. The decreases were slight in some cases, but in others they ran as high as 20, 40, and 60 percent.

In other experiments the lemons were held for several weeks in atmospheres containing 2 or 10 percent of carbon dioxide. In some

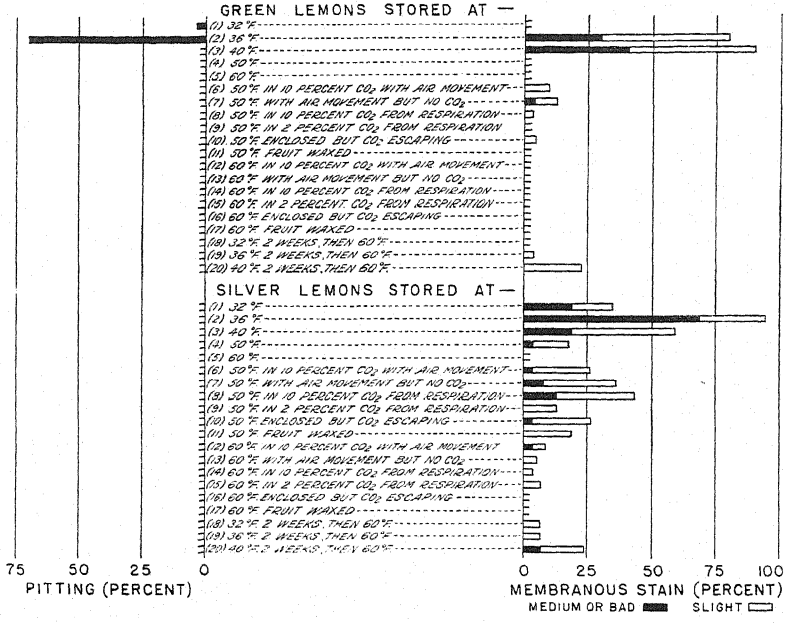


FIGURE 8.—Effect of storage conditions on pitting and membranous stain of lemons stored at various temperatures February 17, 1936; record made 16 weeks later. The carbon dioxide treatments were continued during the first 4 weeks of storage.

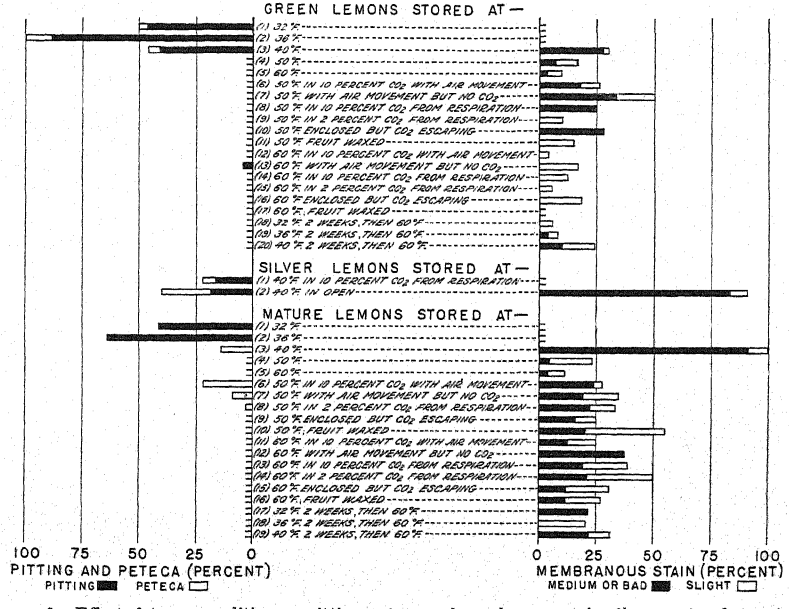


FIGURE 9.—Effect of storage conditions on pitting, peteca, and membranous stain of lemons stored at various temperatures May 12, 1936; record made 14 weeks later. The carbon dioxide treatments were continued during the first 4 weeks of storage.

of these tests the carbon dioxide was built up by the respiration of the fruit, and in others it was obtained by continuous renewal with an atmosphere to which carbon dioxide had been added. The results are shown in figures 5 to 9, inclusive.

In the experiment reported in figure 5, lemons were held at 50° F. in 10 percent of carbon dioxide for 10 weeks without the development of pitting, but neither was there pitting in the control lot in the open.

In the experiments reported in figure 6, the fruit was held at 32° and at 40° F. during 6 weeks' exposure to an atmosphere containing 10 percent of carbon dioxide. No control lot was held at these temperatures for the same length of time as the treated fruit, and it is therefore impossible to draw definite conclusions. There is some indication that the carbon-dioxide treatments caused a decided decrease in the pitting of green lemons but possibly a slight increase in the pitting of silver lemons.

The experiments reported in figure 7 were carried out at 40° and 50° F., and those reported in figures 8 and 9 at 50° and 60°. In all cases control lots were held in similar confinement but in atmospheres that were free from any accumulation of carbon dioxide. In the experiments of figure 7 the carbon-dioxide treatments were continued for 3 weeks and in the experiments of figures 8 and 9 for 4 weeks.

In a total of 24 experiments in which the fruit was exposed to 10 percent of carbon dioxide and in 10 experiments in which it was exposed to approximately 2 percent, there were 30 instances in which no pitting developed on either treated or control fruit. In the experiments in which the fruit was exposed to 10 percent of carbon dioxide there were three instances in which there was more pitting on the control fruit than on the treated lot and but one instance in which the reverse condition held. The results do not give any indication that exposure to low percentages of carbon dioxide had any tendency to cause pitting.

In the 34 carbon-dioxide tests mentioned above there were 4 instances in which peteca occurred and in 3 of these it was worse on the treated than on the control fruit. If peteca is to be considered a form of pitting, this record just balances the favorable contrast reported above, leaving the effect of low percentages of carbon dioxide null.

EFFECT OF OILED WRAPPERS AND WAXING

Lemons that were waxed and those that were packed in heavily oiled wrappers developed less pitting than those stored without these treatments. This is shown in figures 3 to 9, inclusive. When compared with the controls, the treated fruit showed approximately three times as many instances of a definite decrease as of a definite increase in pitting. The results indicate a similar or even greater decrease in the peteca form of pitting on the fruit that received an oiling or waxing treatment.

RED BLOTCH

Red blotch, or adustiosis, is a peel defect that is much shallower and more diffuse than pitting, although in some instances it seems to shade gradually into the milder or shallower type of pitting (p. 798). The diseased area is reddish brown in color, and in early and marginal stages of the disease it appears to be made up of an aggregation of small brown dots, but later a more continuous browning develops.

In the present experiments red blotch has been of relatively rare and rather erratic occurrence. It was not found on lemons that were mature when placed in storage and was far more common on green than on silver lemons. It was found almost exclusively on fruit stored at 36° or 40° F., with an occasional occurrence at 32° and 50° but never at 60°.

Either by chance or otherwise, the lots that were exposed to carbon dioxide remained entirely free of the disease. Fruit that was waxed or held in heavily oiled wrappers developed as much red blotch as the controls.

MEMBRANOUS STAIN

CHARACTERISTICS

Membranous stain, or membranosis, is characterized by a browning or darkening of the membranes or carpellary walls between the segments. This condition is shown in figure 10. The central core tissues and the inner tissues of the rind are also sometimes affected. The disease can be detected only when the lemons are cut.

Membranous stain, especially in its milder forms, was by far the most prevalent disease found in the present experiments. The record of its occurrence is shown in figures 3 to 9, inclusive.

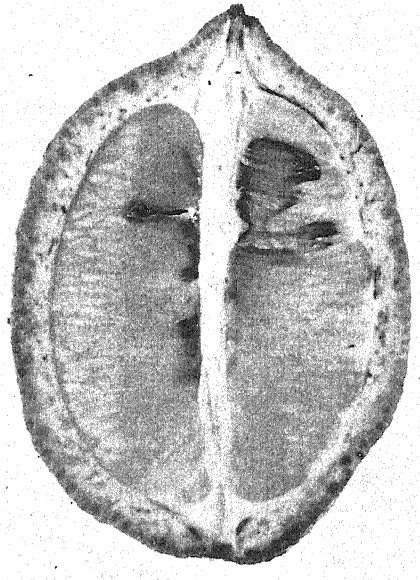


FIGURE 10.—Membranous stain as seen in longitudinal section of a silver lemon that was held 13 weeks at 40° F.

EFFECT OF TEMPERATURE

Membranous stain showed an extreme response to temperature. Lemons stored at 32° F. usually remained practically free from the disease; whereas those held at 40° usually were seriously affected, in many cases but little of the fruit remaining free. In the few tests in which the fruit was held at 31° or 34° the results were similar to those at 32°. There was much less membranous stain at both 36° and 50° than at 40° and far less at 60° than at 36° or 50°, yet the amount of stain at 60° was much greater than at 32°.

In most of the experiments the lemons were held at 70° F. for a week after removal from storage. Membranous stain did not increase during this period. This fact would seem to prove that the elimination of the disease at 32° was permanent and not due to any temporary inhibition of oxidation or related processes.

The extreme temperature response of membranous stain and its sharp divergence from that of pitting make an interesting background for speculation as to the fundamental causes of the two diseases.

With fruit that was moved from one temperature to another the development of membranous stain seemed in general to be in accord with the relative periods at the two temperatures. Fruit held at 32° F. for 1 or 2 weeks before being stored at 50° or 60° usually developed less membranous stain than that held continuously at the higher temperatures, and fruit held at 40° for 1 or 2 weeks before being stored at 50° or 60° usually had more stain than that held continuously at the higher temperatures.

In the experiments reported in figure 3, green and silver lemons that were held at 100° F. for 21 hours before being stored at 40° developed less membranous stain than those that were placed immediately at 40°.

EFFECT OF CARBON DIOXIDE

Increasing the carbon dioxide content of the storage atmosphere decreased the occurrence of membranous stain.

In the experiments in which the lemons were exposed to a high percentage of carbon dioxide for 2 or 3 days before being stored (figs. 3–5) there were 10 instances in which there was more membranous stain in the controls than in the treated fruit and 5 instances in which the reverse was true. In two lots stored at 32° F. the treated lemons had more of the disease than the controls. At this temperature both the treated and control lots remained remarkably free from membranous stain.

With fruit held for several weeks in constantly renewed atmospheres to which 10 percent of carbon dioxide had been added (figs. 7–9) there were 12 instances in which membranous stain was greater on the control fruit and 2 instances in which it was greater on the treated fruit.

Experiments were made in which the carbon dioxide from respiration was allowed to accumulate in the storage chamber. In 11 of these experiments the carbon dioxide was held at approximately 10 percent and in 12 it was held at 2 to 3 percent (figs. 6–9). There were more instances in which the membranous stain in the control fruit exceeded that in the treated fruit than there were of the reverse condition.

The results indicate that the presence of carbon dioxide in the storage atmosphere is beneficial rather than harmful so far as membranous stain is concerned. The favorable effect of exposure to carbon dioxide, together with the favorable effect of low temperature, would suggest the possibility that the control in both cases is due to suppression of oxidation.

The lack of harmful effects from exposure to carbon dioxide does not prove that some form of ventilation or air circulation may not be beneficial. There may be other respiratory products that accumulate to a harmful degree. A comparison of the fruit that was confined, either for carbon dioxide treatment or as controls, with that held in the open (figs. 5–9) shows that there were a few more instances of membranous stain on the confined fruit exceeding that on the fruit in the open than there were of the reverse condition; but the contrast is hardly great enough to suggest a practical significance.

EFFECT OF OILED WRAPPERS AND WAXING

Waxing the lemons tended to decrease the development of membranous stain (figs. 3, 4, 6–9). At 32° and 60° F. little membranosis developed on either treated or control lots. When compared with the controls, the treated fruit showed approximately four times as many instances of a distinct decrease as of a distinct increase in

membranous stain at 40° and 50°. The few experiments with heavily oiled wrappers gave results that were favorable rather than otherwise.

Waxing and the use of heavily oiled wrappers would be expected to increase the carbon dioxide and decrease the oxygen content of the internal atmosphere of the fruit, and it is possible that their favorable effect is to be explained on the same basis as that of the carbon dioxide treatments.

WATERY BREAK-DOWN

CHARACTERISTICS

In low-temperature storage the lemons sometimes became water-soaked, soft, and spongy, as if frozen. Both the peel and the pulp were usually involved. The term "watery break-down" is suggested for this diseased condition. A similar disease has been reported in grapefruit.[6]

STORAGE RESPONSE

The disease was confined to lemons held for long periods in storage at 31° or 32° F. In some cases 25 percent or more of the fruit was affected at the time of removal and there was usually an equal or higher percentage of the fruit that became watery when held for a few days after removal. Green lemons remained relatively free of the disease, but silver and mature lemons became badly affected. Lemons that were exposed to high temperature or to high percentages of carbon dioxide before being stored at 32° had far more of the disease than those that did not receive these treatments.

Watery break-down is evidently a low-temperature suffocation type of disease.

SCALD

The term "scald" is sometimes used for injuries resulting from chemical treatments or exposure to high temperature. In the present experiments scaldlike injuries were occasionally found in low-temperature storage. With green lemons, the disease was characterized by a general disappearance of the green color and a slight depression of the affected peel. With silver and mature lemons no depression was evident, and the diseased condition was indicated by a color change of the peel from normal yellow to a honey yellow or cinnamon buff.[7] The trouble was sometimes associated with watery break-down and is probably closely related to that disease, yet no instance was observed in which one disease developed into the other.

INTERNAL DECLINE

Internal decline, or endoxerosis, was found occasionally in the present experiments, but with little if any reference to storage conditions. The disease is characterized by a breaking down and drying of the internal tissue, particularly that near the stylar end.

FIRMNESS AND COLOR

Notes were taken on the effects of the different treatments upon the color and firmness of the fruit, and the results were recorded as percentages. Lemons showing ideal firmness were given a rating of 100 and softer lemons a lower rating. Fruit that had the most desirable color for immediate marketing was rated at 100; that which was

[6] BROOKS, C., and MCCOLLOCH, L. P. SOME STORAGE DISEASES OF GRAPEFRUIT. Jour. Agr. Research 52: 319-351, illus. 1936.

[7] RIDGWAY, R. COLOR STANDARDS AND COLOR NOMENCLATURE. 43 pp., illus. Washington, D. C. 1912.

greener was given a lower rating and that which had too much brown in the yellow was given a higher rating. The results from two of the experiments are shown in figures 11 and 12. The results from the other experiments were in general agreement with these but showed slightly less contrast.

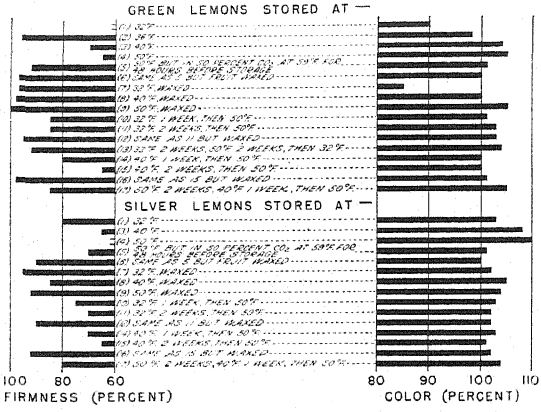


FIGURE 11.—Effect of storage conditions upon the firmness and color of lemons. Green and silver lemons stored March 20, 1934; record made 12 weeks later.

The effects upon color were more pronounced with green lemons than with silver lemons. The rate of color development usually increased with an increase in the storage temperature.

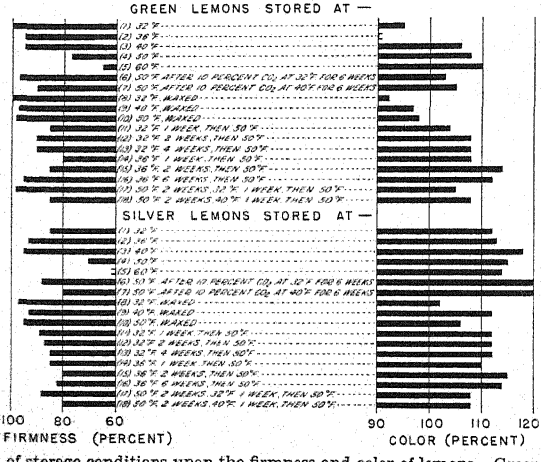


FIGURE 12.—Effect of storage conditions upon the firmness and color of lemons. Green and silver lemons stored January 23, 1935; record made 16 weeks later.

Carbon dioxide treatments usually delayed color development, especially in the prestorage treatments with high percentages of carbon dioxide.

Color usually developed more slowly in waxed than in unwaxed fruit.

The firmness of the fruit usually decreased with a rise in the storage temperature. Fruit held for 1 to 3 weeks at a lower temperature before being moved to a higher usually showed decidedly greater firmness than that held continuously at the higher temperature.

Carbon dioxide treatments had little, if any, effect upon loss of firmness.

Waxing had a most pronounced effect in delaying the loss in firmness, especially at the higher temperatures.

In the tests reported in figure 11 several of the waxed and unwaxed lots were weighed before and after being stored, and it was found that the loss in weight of the waxed fruit was 75 to 95 percent less than that of the control fruit.

SUMMARY

Certain storage diseases of lemons are described and experiments are reported showing the response of these diseases to storage conditions.

The storage lots were small but great care was taken in selecting them.

Fruit was held in storage at 32°, 36°, 40°, 50°, and 60° F.

During the first 6 or 8 weeks of storage there was more decay at the higher temperatures, but with longer storage this was sometimes reversed, apparently because of decay following watery break-down and pitting.

Carbon dioxide failed to check alternaria decay.

Pitting did not occur in 60° F. storage and was seldom serious at 50°, but was the great limiting factor in storage at lower temperatures. It was much worse at 32° and 36° than at 40°.

Holding the lemons at 32°, 36°, or 40° F. for 1 or 2 weeks before storing them at a higher temperature did not increase pitting.

Prestorage treatments with high percentages of carbon dioxide tended to decrease pitting.

Waxed lemons developed less pitting than unwaxed ones.

Membranous stain showed most pronounced temperature response. Its occurrence at 32°, 36°, 40°, 50°, and 60° F. stood approximately in the order of 1, 12, 22, 8, and 2 percent respectively, with usually only a small amount of the disease at 32° and 75 to 100 percent of the fruit affected at 40°.

Fruit held at 32° F. 1 or 2 weeks before being stored at 50° or 60° usually developed less membranous stain than that held continuously at the higher temperature, but when held at 40° 1 or 2 weeks before being stored at 50° or 60° it sometimes developed more.

Carbon dioxide gas storage decreased membranous stain. This was true with fruit receiving prestorage treatments with high percentages of carbon dioxide and with that exposed for several weeks to low percentages produced either by the addition of carbon dioxide to the atmosphere or by building it up by means of the fruit respiration.

Waxed fruit usually had less membranous stain than unwaxed.

Watery break-down was sometimes serious in 32° F. storage but did not occur at higher temperatures. Scald and red blotch were occasionally found on fruit held at low temperatures.

Prestorage treatments with high percentages of carbon dioxide tended to delay color development.

Waxed fruit lost weight and firmness very much more slowly than unwaxed fruit.

No injury resulted from the accumulation of low percentages of carbon dioxide in the storage atmosphere, and none from prestorage exposure to high percentages of carbon dioxide.

PRODUCTION OF SYNTHETIC MYCORRHIZA IN THE CULTIVATED CRANBERRY[1]

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INTRODUCTION

Investigations in the Ericaceae have played an important role in the broader study of relationships existing between mycorhizal fungi and their host plants. Many species of the family, particularly those growing in peat and raw humus soils, regularly have mycorhiza of a rather uniform type, in which the epidermal or cortical cells of the roots are filled with compact masses of fungus mycelium. According to some investigators, the mycorhizal fungi invade above-ground organs as well as roots of the host, the mycelium in this case being generally distributed throughout the plant in a highly attenuated and sparsely developed form. Extraordinary significance has been ascribed to this systemic form of infection, as the fungus is considered to furnish an indispensable stimulus to root production by the host plant. Other investigators have questioned the existence of systemic infection and its obligate relation to root production. Some workers suspect that the mycorhizal fungus supplies nitrogen to its host, either by making nitrogenous compounds in the peat available or by directly fixing atmospheric nitrogen; others doubt whether the host derives any benefit from its endophyte. The present paper adds to this general subject some observations on the mycorhiza of the cultivated cranberry, *Vaccinium macrocarpon* Ait.

REVIEW OF LITERATURE

A critical review of the literature is essential to an understanding of the discordant views held by present investigators. Stahl in 1900 (*32*)[2] grew seedlings of *Vaccinium myrtillus* in sterilized soil and reported that they were mycorhiza-free but developed as well as mycorhiza-infected plants. Ternetz in 1907 (*37*) reported that several strains of a fungus described as *Phoma radicis*, isolated from roots of different ericaceous species, were able to fix atmospheric nitrogen. The identity of the isolated strains with the root endophytes was not established because mycorhiza-free plants could not be secured for inoculation tests, even by growing plants from surface-sterilized seed planted in peat sterilized at 120° C. on 2 successive days. Ternetz reasoned that the mycorhizal fungus must have been present in the seed, and in confirmation reported finding brown hyphae, similar to those occurring on ericaceous roots, in seed coats of *Calluna vulgaris*, one of the plants with which she worked. After considering various ways in which the mycelium might have reached the seed, Ternetz inclined toward the view that infection takes place during bloom.

[1] Received for publication Feb. 3, 1937; issued February 1937.
[2] Reference is made by number (italic) to Literature Cited, p. 834.

In a series of publications beginning in 1911, Rayner gradually developed the hypothesis of systemic infection and obligate symbiosis in the Ericaceae. Her first (joint) paper (*23*) suggested that mycorhiza might be an important factor in accounting for the natural distribution of *Calluna vulgaris*. Working with the same plant, she reported in 1913 (*16*) finding seed-coat infection in unopened capsules and isolated a fungus from surface-sterilized mature seed which "has since then been identified with the species occurring as mycorhiza in the roots" (*16, p. 70, footnote*). Fungus-free cultures of seedlings were obtained when the strength of the sterilizing solution (mercuric chloride) was increased to 1 percent. Rayner interpreted this to mean that the seed-coat-inhabiting mycorhizal fungus had been effectively eliminated and that in consequence these seedlings were truly mycorhiza-free. All such seedlings failed to produce normal roots. In 1915 (*17*) she reported success in stimulating similar "mycorrhiza-free" seedlings to form roots and grow normally on an agar substrate by inoculating the cultures with a *Phoma* isolated from unopened fruits. The circumstance of root stimulation was considered to prove not only that the *Phoma* was identical with the *Calluna* endophyte but also that the relation between root formation and mycorhizal infection was obligate. As will be pointed out later, the *Phoma* did not produce typical mycorhiza in the inoculated seedlings. Unlike Ternetz, Rayner was of the opinion that the fungus reached the seed by growing up through stem and fruit tissues and that seedlings subsequently became infected by hyphae from seed coats growing into roots shortly after germination. Microscopic examination revealed fine hyphae in leaves and stems of the plant. The final link in the development of Rayner's hypothesis appeared in 1922 (*19*), when she referred her *Phoma* to the nitrogen-fixing *P. radicis* of Ternetz and, by accepting the identity of the fungi, considered that her findings confirmed and extended those of Ternetz.

Christoph in 1921 (*6*) first seriously questioned Rayner's work. He isolated a nonfruiting fungus from roots of *Calluna vulgaris*, which produced typical mycorhiza when inoculated into the soil in which *Calluna* seedlings and cuttings were growing. The fungus was obviously not *Phoma radicis*. Uninoculated plants grew normally and remained free from mycorhiza, in some cases for more than 2 years. Christoph started his plants in sterilized soil, but afterward grew them in open pots unprotected from contamination. Rayner (*18*) promptly replied to this article by criticizing Christoph's work on the three grounds that the seeds were incompletely sterilized, that they were not grown under aseptic conditions, and that the microscopic technique employed in making the examination for infection was inadequate. She charged that Christoph completely overlooked the "fine mycelium" type of infection, which was undoubtedly present in his uninoculated plants. On her part, Rayner minimized the fact that Christoph's fungus produced the "knot" form of mycorhiza in roots of plants growing in previously sterilized soil.

Dufrénoy (*8*) in 1917 reported that the endotrophic mycorhizal fungus associated with root tubercles of *Arbutus unedo* extended throughout all organs of the plant in that species. After death of sepals, petals, and leaves, the fungus continued to grow saprophytically and in that stage produced conidia. The fungus was also said to sporulate on rootlets which it had previously parasitized. By

implication the fungus was considered to be a strain of *Phoma radicis*. It was not isolated from the plant. Working with the same host species, Rivett in 1924 (*26*) concluded that the tubercles were primarily caused by a fungus growing ectotrophically and that the "knot" form of endotrophic mycorhiza developed in them somewhat later. Nontuberculate roots were frequently infected with the attenuated form of hyphae. It was assumed that the three types of mycorhiza—the ectotrophic and both endotrophic forms—were caused by a single fungus, although no isolations were made.

A reinvestigation of the whole problem of mycorhiza in *Calluna* was reported by Rayner (*20*) in 1925. Essentially the same conclusions were reached. Some new details regarding distribution and time of development of mycelium in the plant were given. She stated that "fungal infection and the stimulus to development associated with it on the one hand, and the formation of root mycorhiza on the other hand, must be regarded as distinct phenomena" (*20, p. 285*), although the same fungus was believed to bring about both conditions. When Rayner repeated Christoph's experiment of growing cuttings in sterilized peat, she found that after 8 months the roots appeared to be free from mycelium; i. e., like Christoph's check plants, they failed to develop the "knot" type of mycorhiza. However, she insisted that the attenuated form of the endophyte was present.

In 1928 Doak (*7*) briefly reported isolating a mycorhizal fungus from roots of *Vaccinium corymbosum* and *V. pennsylvanicum*, which produced mycorhiza in "sterile seedlings." Normal root and stem development did not depend on the presence of the fungus. The fungus resembled *Rhizoctonia*. Unfortunately, a complete account of these investigations has not been published.

Using a culture of *Phoma radicis callunae*, isolated by Rayner in 1924, Jones and Smith (*12*) in 1928 reported that the fungus fixed a small amount of atmospheric nitrogen but exhibited unmistakable growth response when available nitrogen was added to the culture solution. The authors felt that the study ought to be repeated with a newly isolated strain of the fungus.

The notion of systemic infection was carried still further by Rayner in 1929 (*21*), when she reported that in the genus *Vaccinium* the endophyte penetrates into the endosperm of the developing seed. The investigation dealt largely with *V. macrocarpon* and *V. oxycoccus*, though ovarial infection by fine hyphae was reported for seven species of the genus. Fruit infection was detected as early as July. In mature seed the embryo alone lacked infection. When seed were aseptically transferred to agar from surface-sterilized berries, the fine hyphae in the seed coat and endosperm became active and penetrated into the developing hypocotyl but did not grow into the agar, a circumstance that appeared "very puzzling" (*21, pp. 60–61*). Despite the fact that the endophyte was never isolated from any part of the cranberry plant, it was assumed that the mycorhizal fungus was a strain of *Phoma radicis*. Seedlings were grown on agar with and without seed coats removed. In both cases they grew well but rooted irregularly, and roots in contact with the agar browned early. Roots and stems of the seedlings were found to be sparsely infected with fine hyphae. It was argued that since the endosperm is infected the seedlings likewise must be infected; consequently, it is impossible to

follow the classic method of inoculating fungus-free plants with a pure culture of the endophyte in *Vaccinium* species. Concerning the failure of the seedlings to develop the "knot" type of mycorhiza in agar cultures, the following statements appear (*21, pp. 66–68*):

> [Mycorrhiza formation] is an annual event, influenced largely by soil conditions. * * * The direct relation of mycorrhiza formation to soil constitution is emphasized once more, and in *Vaccinium* there can be little doubt of its immediate connexion with an abundant supply of natural humus. * * * Mycorrhiza formation [in *Vaccinium*] is likewise hindered by certain conditions in the rooting medium. * * * the formation of mycorrhiza is partially inhibited in the roots of both species [*Vaccinium* and *Calluna*] when grown in a sterilized medium.

In 1929 Knudson (*13*) reported growing seedlings of *Calluna vulgaris* for more than 2 months without the formation of mycorhiza. The plants rooted normally. Seed were surface-sterilized with calcium hypochlorite solution and planted on nutrient agar in test tubes. The agar slants remained sterile except one series in which the seed contained a considerable amount of chaff. All the cultures in this particular series developed an *Alternaria*, which was unable to penetrate into the roots. *Phoma* did not appear in any of Knudson's cultures, and no attempt was made to isolate the endophyte from roots of naturally infected plants.

Rayner immediately criticized Knudson's work, claiming that he used imperfect sterilization methods and then overlooked infection in his seedlings because of faulty technique. She stated (*22, pp. 383–384*):

> In imperfectly sterilised cultures [i. e., those in which the *Phoma* presumably occurring in the seed coats is not killed] in an aseptic rooting medium typical mycorrhiza is not formed; mycelium is casual in distribution, often very sparsely developed, and may be extremely difficult to put in evidence in the earlier stages of growth. * * * The early stages of infection in cultures such as those described show no hyphal complexes within the root cells * * *. The two phenomena—infection of the seedling at germination and the formation of mycorrhiza—are distinct; the former is invariable, the latter conditioned by the nature of the rooting medium * * * Presumably, by "root infection" Knudson understands the formation of the characteristic hyphal complexes found in typical *Calluna* mycorrhiza. In my experience, these are never formed in a sterile agar rooting medium of the kind used * * *. Knudson's conclusions tend to confirm those reported by Christoph because he has employed a similar technique and does not appreciate the true character of seedling infection as distinct from mycorrhiza formation.

Further support for the nitrogen-fixing ability of *Phoma radicis callunae* was reported in 1929 by Rayner and Smith (*24*). In the course of a physiological investigation of the fungus it was found that both the fungus and *Calluna* seedlings thrived better when nitrates and other forms of combined nitrogen were left out of the nutrient media. Strains of the endophyte were said to exhibit a high degree of specialization toward their respective host plants. On the subject of mycorhiza formation it was again stated (*24, p. 287*) that "(* * * roots although infected by mycelium do not develop functional mycorrhiza in a sterilised substrate)."

Investigating the nutrient requirements of the cranberry (*Vaccinium macrocarpon*) in 1931 and 1932, Addoms and Mounce (*1, 2*) concluded that the mycorhizal fungus alone was not able to supply the plant's demand for nitrogen. Rooted cuttings were grown in sand in open pots. Fungus mycelium, presumably of *Phoma radicis*, was observed in above-ground plant parts, including fruit and seed, as well

as in roots. There appeared to be a positive correlation between the amount of mycelium present and vigor of growth in the host plant. Isolation of the fungus was not attempted.

Two important contributions to the subject appeared in 1933. Knudson (*14*) repeated his experiments with *Calluna* with greater precision and presented new evidence that calcium hypochlorite effectively sterilized the seed. Using Rayner's microscopic technique, he was again unable to detect mycelium of any kind in seedlings grown on agar. Root formation was completely inhibited on such agar substrates as potato dextrose and those containing peptone, but seedlings rooted normally on favorable media, i.e., the rooting response was obviously controlled by the composition of the substratum. Again *Phoma* failed to appear in any of the cultures. Friesleben (*9*) isolated from the roots of *Vaccinium myrtillus* a mycorhizal fungus that reproduced the "knot" type of mycorhiza in seedlings grown from surface-sterilized seed. The fungus, which failed to fruit, was tentatively called *Mycelium radicis myrtilli*. The plants were grown in a peat-sand mixture, in flasks that were sterilized and handled aseptically throughout the experiment. Employing Rayner's technique, Friesleben was unable to find the attenuated form of infection in his "sterile" plants. In one important respect his results confirmed those of Rayner, namely, that seedlings in the fungus-free flasks failed to produce roots and soon died.

Using the same technique as before, Friesleben (*10*) in 1934 isolated four root fungi from three *Vaccinium* species (two strains from *V. myrtillus* and one each from *V. vitis-idaea* and *V. uliginosum*), each of which induced formation of true mycorhiza not only in its original host plant but also in the other three species of *Vaccinium* and in *V. oxycoccus* as well. Stimulation to root and shoot growth accompanied synthesis in all cases. None of the fungi fruited. Still more important, Friesleben found that the stimulus to root formation in sterilized peat was brought about equally well by several nonmycorhiza-forming fungi isolated from soil surrounding roots and from surfaces of vegetative parts of native plants. A culture of Rayner's *Phoma radicis callunae* parasitized *V. oxycoccus* but reacted toward *V. vitis-idaea* in the same manner as the other nonmycorhiza-forming fungi. These discoveries compelled Friesleben to abandon the idea of specific and obligate symbiosis in *Vaccinium* and to hold instead that root suppression is due to an unfavorable condition in the rooting medium, which may be corrected by the presence of various fungi whether or not they are mycorhiza forming. He pointed out that fungi might alter the substratum either by secreting substances needed by the higher plant or by breaking down toxic substances produced during sterilization.

A comprehensive series of experiments with ericaceous mycorhiza was reported by Friesleben in 1935 (*11*). Seedlings of 21 species in 13 genera, representing all except 2 tribes of the Ericaceae, were grown in sterilized peat, and except for the tribe Arbuteae all the species exhibited growth repression similar to that found earlier (*10*) in *Vaccinium* species. The repressive effect disappeared when the sterilized peat was inoculated with living cultures either of the root fungi previously isolated from *Vaccinium* spp. (*10*) or of certain other nonmycorhiza-forming soil fungi. Mycorhiza was produced by the *Vaccinium* endophytes in most of the species. Seedlings in asymbiotic

cultures on favorable nutrient agar rooted and grew well, but not quite so vigorously as in inoculated sterilized peat. On the other hand, repressive effects similar to those of sterilized peat developed in cultures on the following media: (1) Ether, alcohol, and water extracts from peat; (2) agars containing peptone, malt extract, and potato; and (3) extracts from fungus mycelium. Friesleben concluded that the stimulating effect of fungi on heath plants is brought about by inactivation, destruction, or absorption of repressive substances in the rooting medium, rather than by secretion by the fungi of substances stimulating to the higher plant.

In the mass of conflicting evidence and opinion outlined above, investigators have agreed that ericaceous plants generally possess mycorhizal fungi in a form variously referred to as "hyphal complex," "knot," or "Hyphenknäuel," because of the typical structures produced by the fungi in root cells. Fungi isolated from these "hyphal complexes" have been found to reproduce similar structures under experimental control in *Calluna vulgaris* (by Christoph), *Vaccinium corymbosum* and *V. pennsylvanicum* (by Doak), and *V. myrtillus*, *V. vitis-idaea*, and *V. uliginosum* (by Friesleben). Friesleben's *Vaccinium* endophytes also produced the hyphal complex type of mycorhiza in 10 additional genera of Ericaceae, including *C. vulgaris*. The endophytic fungi isolated by these investigators failed to produce spores when grown in pure culture. Plants grown in the absence of the endophytes apparently developed normally when certain other necessary conditions were provided.

On the contrary, there has been lack of agreement regarding the role attributed to *Phoma radicis*. Rayner's views have been repeatedly and clearly set forth, as indicated by excerpts in the foregoing review. Two of the fundamental claims she makes for the fungus have been placed in a doubtful category by other workers, since (1) root suppression has been attributed (by Knudson and by Friesleben) to factors inherent in the rooting medium rather than, as Rayner postulates, to lack of *Phoma* in the plants and (2) the actual existence of systemic infection in the form of "fine hyphae" has been openly questioned. Confirmation of the latter phenomenon has come only from observers (Dufrénoy, Rivett, Addoms and Mounce) who have examined material microscopically but have not attempted to grow the fungus and host apart from one another. Those who have isolated endophytes from roots (Christoph, Doak, and Friesleben) or who have grown plants for the specific purpose of determining whether mycorhiza is indispensable (Stahl and Knudson) have not confirmed it. The widely circulated view that *P. radicis* is the fungus causing true mycorhiza in the Ericaceae is not based on experimental proof, since every strain of *Phoma* so far isolated has failed to produce the hyphal complex form of mycorhiza under controlled conditions (Ternetz; Rayner; and Friesleben (*10, p. 449*)).

SCOPE OF THE PRESENT INVESTIGATION

The present paper reports isolation of root endophytes from four ericaceous species—*Vaccinium macrocarpon* Ait., *V. canadense* Kalm., *Chamaedaphne calyculata* (L.) Moench, and *Ledum groenlandicum* Oeder.—and a method of synthesizing mycorhiza in a sterilized agar medium. Although the endophytes from the four host plants have

not fruited, marked growth differences in culture leave little doubt that the fungi are specifically distinct, and none of them resembles *Phoma radicis* as described in the literature or as compared with a culture of *P. radicis callunae* Rayner, obtained from the Centralbureau voor Schimmel Cultures in Baarn, Netherlands. Originating from three different host genera, all four fungi produce hyphal complex infections in roots of cranberry seedlings (*V. macrocarpon*) grown on agar under pure-culture conditions. The freedom with which the fungi penetrate roots, as well as the rooting response of seedlings, is shown to be markedly influenced by the constitution of the substrate.

ISOLATION OF THE ENDOPHYTES

The cranberry endophyte was isolated from roots of seedlings grown in the greenhouse in Washington, D. C., in peat brought from a commercial cranberry planting in Wisconsin the previous fall and kept moist until used. Seeds were planted in January and February 1932. Seedlings started emerging about 3 weeks after planting, and a month later some of the roots on most plants were typically infected with the hyphal complex form of mycorhizal fungus.

Isolations were made without previous surface sterilization of the roots. Roots were thoroughly washed in sterilized water with a soft camel's-hair brush and layers of epidermis one cell in thickness were stripped from them with No. 12 steel needles. After the positions of favorably located infected cells had been carefully noted under the compound microscope, the strips were transferred to a watch glass, where the epidermal tissue was further dissected into pieces of some half dozen cells or less, containing a minimum number of infected cells. Five or six of these pieces were transferred to the surface of a thin layer of nutrient agar on a cover glass, covered with a small drop of clear water agar, and then inverted over a Van Tieghem cell with a drop of sterile water in the bottom. In the most favorable preparations the pieces of epidermis lay parallel to and near the cover glass, completely embedded in agar, so that growth from the mycorhizal cells could not only be watched under a 4-mm objective but could also be photographed in situ with a fair degree of success.

Out of a large number of slides prepared in this manner, most of the tissue pieces remained sterile, a few were evidently contaminated, and 16 yielded pure cultures of the endophyte. As observed under the microscope, the first indication of growth in the mycorhizal cells was an increase in the mass of internal hyphae, sometimes to the extent of distending the cell walls considerably before the hyphae broke through. Some 40 hours or so after the cultures were started, hyphae began to pierce the walls and to grow into the agar at an extremely slow rate. The slides were held under observation for at least 6 days, after which the developing fungus growths were transferred to test tubes of nutrient agar. Plate 1, *A* to *C*, illustrates the development of hyphae in one such preparation that contained three "mycorhizal cells" (indicated as *a*, *b*, and *c*). In plate 1, *A*, photographed 44 hours after the slide was prepared, a hypha had just emerged from cell *a*; there was no growth from the other two cells. In *B*, 92 hours old, a second hypha had made its appearance, coming from cell *b*. *C* shows the condition at 139 hours, with cell *c* greatly swollen by the development of internal hyphae. A short time later, numerous hyphae grew out

from cell *c* and the entire piece was transferred to a test tube. *D* shows another preparation photographed when 6 days old, in which several hyphae had emerged from the torn end of the single mycorhizal cell.

The same method of isolation was followed with the other three host species except that, instead of using roots of seedlings, mature plants were dug, all fine rootlets were removed, and the plants were reset in the same soil. Isolations were made from newly formed rootlets as soon as mycorhiza formation was evident. This part of the work was carried out during the summer of 1932 in Wisconsin, where equipment was lacking to make photographic records; however, the development of hyphae was very carefully noted under the high powers of the microscope. *Vaccinium canadense* gave exceptionally satisfactory preparations, as the infected epidermal cells separated easily and two transfers of completely free single cells were obtained, each of which yielded a culture of the fungus. A total of 8 isolations of the *V. canadense* endophyte, 14 of the *Chamaedaphne* endophyte, and 2 of the *Ledum* endophyte were secured. All fungi from each host plant were identical in culture.

CULTURAL CHARACTERISTICS

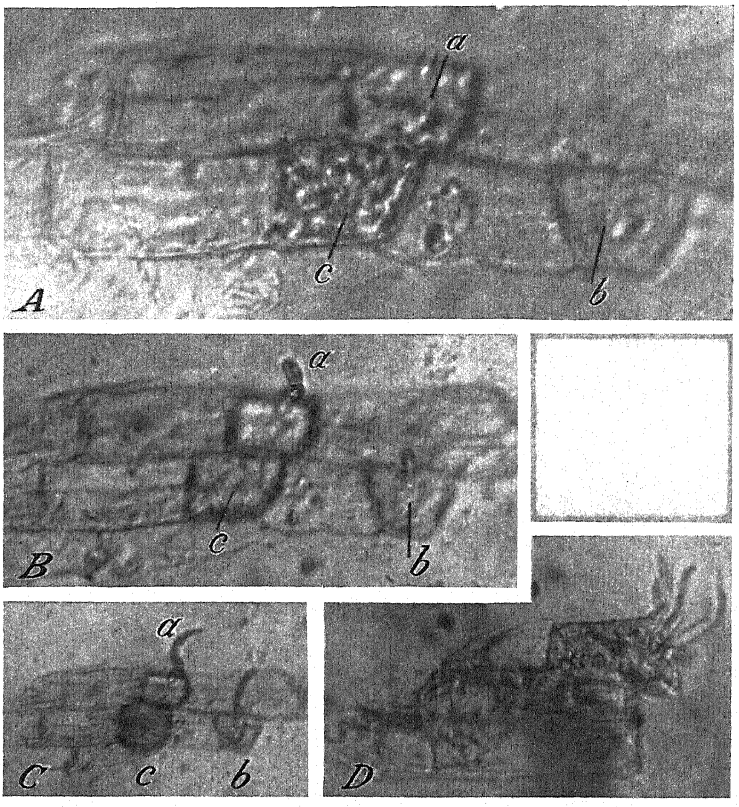
The cultures were subsequently grown on a wide variety of media, including most of the common laboratory sorts, and were subjected to various light and temperature conditions in unsuccessful attempts to induce sporulation. Aside from the fact that all four fungi have septate hyphae, no morphological characters have been developed that would help to determine their systematic relationships. The most prominent characteristic of the fungi as a group is their extremely slow rate of growth, the cranberry fungus having a radial growth of about 0.25 mm per day at room temperature on most media; the blueberry (*Vaccinium canadense*) fungus, about 0.10 mm; the *Ledum* fungus, about 0.20 mm; and the *Chamaedaphne* fungus, about 0.40 mm.

Individual hyphae of the cranberry fungus are invariably hyaline. On some media they remain relatively slender and separate, with the definitely self-limiting colony submerged (pl. 2, *A*, *c*), and they are cartridge buff [3] in color. On other media, notably on onion and beer-wort agars, the colony becomes semistromatic with cells greatly swollen and densely packed with oil globules, the fungus tissue eventually piling up on the surface and breaking into numerous folds and fissures (pl. 2, *B*, *c*). In the latter condition and in older cultures on some other media the colony assumes a chamois or Natal-brown color.

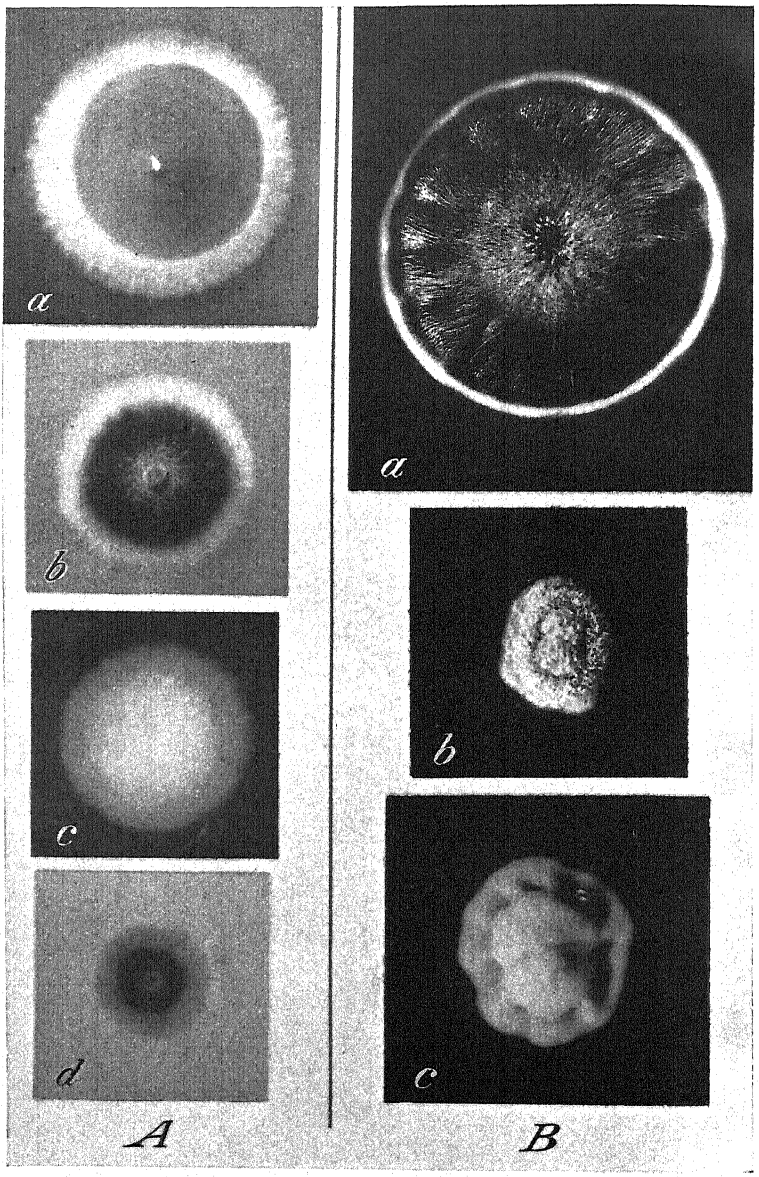
The blueberry (*Vaccinium canadense*) fungus presents an extreme contrast. Here the colony is dark ivy green to olivaceous black, and the individual hyphae are smoky, even showing a dark tinge in hyphal walls when forming "knots" in the lumina of cranberry root cells. The colony does not grow as large as the cranberry fungus (pl. 2, *A*, *d*). The major portion of the colony is submerged, but it is usually covered with a velvety layer of erect aerial hyphae about 0.5 mm in length. Oil globules occur in abundance on most media.

The *Chamaedaphne* endophyte has hyaline hyphae that on some media become dark-colored with age. The colony on most media is dark-colored, and a thin layer of aerial hyphae above darker sub-

[3] Color names used are according to Ridgway (*85*).



Cranberry mycorhizal fungus isolations. *A*, Culture No. 24, 44 hours after being placed in hanging drop of agar. The eight-celled piece of root epidermis contains three hyphal complex cells (*a*, *b*, *c*). A hypha has emerged from cell *a;* no growth from cells *b* and *c*. × 750. *B*, Culture No. 24, 92 hours old. Hypha at *a* has elongated, a second hypha has emerged from cell *b*, and cell *c* is beginning to enlarge from internal growth of hyphae. × 500. *C*, Culture No. 24, 139 hours old. Cell *c* is greatly enlarged by internal growth of hyphae. × 270. *D*, Culture No. 25, 6 days old. Hyphae emerging from torn end of single mycorhizal cell. × 240.



Mycorhizal fungus colonies: *A*, 44 days old on modified McArdle agar; *B*, 47 days old on onion agar; *a*, *Chamaedaphne* endophyte; *b*, *Ledum* endophyte; *c*, cranberry endophyte; *d*, blueberry endophyte. Note particularly the difference in type of growth between *a* and *b* on onion agar and *a* and *b* on McArdle agar. × 1½.

merged mycelium produces a mass effect of deep olive gray to olivaceous black. On some media the color is deep mouse gray, with a margin almost colorless (pl. 2, *A*, *a*, and *B*, *a*). The hyphae penetrate more deeply into the agar than do the hyphae of the two fungi just discussed, and in test tubes the colony eventually covers the entire agar surface; that is, it is not self-limiting. Oil is not ordinarily formed in the hyphae.

The *Ledum* fungus hyphae are hyaline at first and remain so on some media while becoming dark with age on others. The colony is mummy brown to fuscous black practically to its margin. On most media the mycelium is submerged, but occasionally aerial tufts of pale gull-gray to dark olive-gray hyphae are produced (pl. 2, *A*, *b*, and *B*, *b*) The colony rarely extends to the edge of the agar surface. Oil is formed abundantly on some media and is entirely lacking on others. The *Ledum* and *Chamaedaphne* endophytes bear more resemblance to each other than to any of the other fungi, but exhibit well-defined differences on every medium so far used (pl. 2, *A* and *B*).

The cultures have been maintained since 1932 on a modification of the agar used by McArdle (*15, p. 299, footnote*), made up as follows:

	Grams		*Grams*
Agar	15 to 20	$Ca(NO_3)_2$	0. 50
Maltose	2. 0	KH_2PO_4	. 25
Dextrose	2. 0		*Cubic centimeters*
Soluble starch	2. 0		
$MgSO_4$	. 50	H_2O (distilled)	1, 000

To this formula a small amount of peptone is sometimes added, to the advantage of the fungi but spoiling the usefulness of the culture as a source of inoculum because of the repressive effect of the peptone on seedling cranberry roots. Various sugars have been satisfactorily substituted for those named, and ammonium sulphate $(NH_4)_2SO_4$, may partly or completely replace the calcium nitrate, $Ca(NO_3)_2$, as a source of nitrogen.

SYNTHESIS EXPERIMENTS WITH THE CRANBERRY MYCORHIZAL FUNGUS

METHODS AND OBJECTIVES

After numerous attempts had been made to obtain root infection in cranberry seedlings growing in agar, the following method was found to give consistently good results: Sterile seed was planted in test tubes on 0.85- or 1-percent agar, made with distilled water, to which no nutrients of any kind were added. Sterile seed was obtained either by transferring seed directly from surface-sterilized sound berries or by treating cleaned seed with calcium hypochlorite solution (*10, 14*, etc.) for 30 minutes or longer. In this connection it may be mentioned that cranberry seed germinate with much greater regularity if permitted to remain in the fruit until January at a temperature sufficiently low to keep the berries in prime condition (*4, p. 10*). Both methods of sterilization of seed fail to meet Rayner's view that it is not possible to eliminate endosperm-borne mycelium of *Phoma radicis* from the seeds. This matter is considered at length in a later section, but it may be pointed out here that literally hundreds of plantings have been made on water agar and on nutrient agars

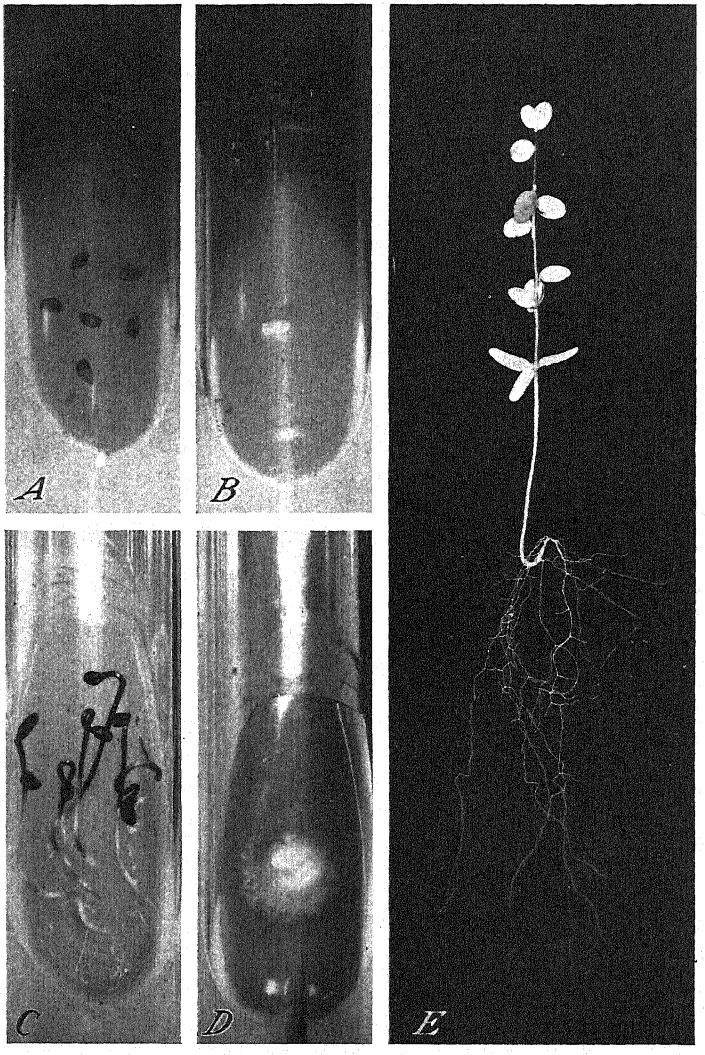
favorable to the growth of the cranberry root endophyte without once encountering *Phoma radicis* in culture (pl. 3, *A* to *D*).

As the seed germinates the roots penetrate the agar freely and in most cases remain slender and bright-colored, branch naturally, and appear to be normal and healthy in all respects. The plants grow for 3 or 4 months without showing marked signs of nutritional deficiences, reach an average height of about 4 cm, and produce six or more true leaves. At the end of the period they differ from plants grown in soil mainly in having somewhat longer internodes, fewer and slightly smaller leaves, and a much reduced root system. If transplanted to soil from water agar while actively growing, the seedlings will thrive as well as soil-planted seedlings of the same age. In the tubes the plants may remain alive and green for a year provided the agar does not dry out. Exposed roots redden in bright sunlight. Plate 3, *E*, illustrates an average 16-weeks-old plant grown in water agar.

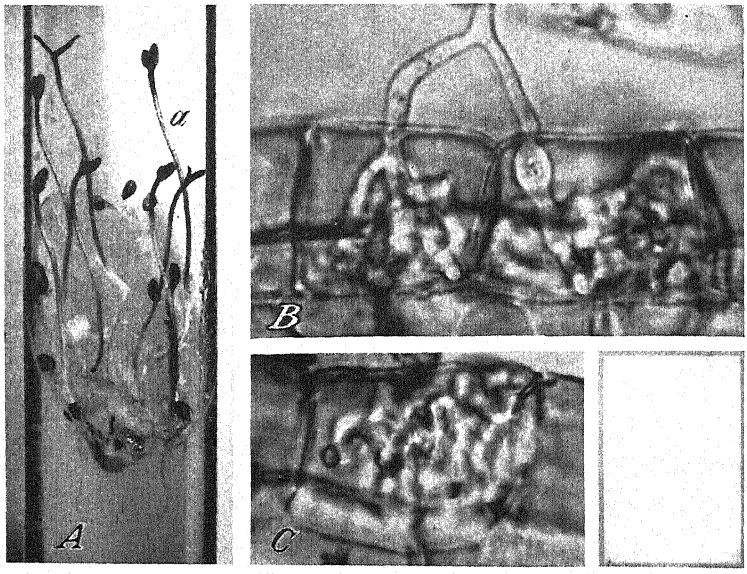
An occasional lot of distilled-water agar proves to be somewhat unsatisfactory. Here the roots cease growing, after penetrating a short distance into the agar, and send out numerous abortive branches, and the entire root system loses its bright, clear color, turning brown or black. The aerial portion of the plant is not affected, although there is a tendency to produce adventitious roots above the agar. This is apparently a mild form of toxicity due to the medium. More severe cases of toxicity brought about by adding various substances to the agar are discussed later.

The seedling cultures may be inoculated with the mycorhizal fungus at the time the seed is planted, or if it is preferred to test the sterility of the seed the operation may be delayed until after the seed has germinated. Inoculations are made simply by spreading liberal quantities of crumbled fungus tissue from stock cultures (p. 819) among the seeds or plants (pl. 4, *A*). Hyphae from each piece of inoculum grow remarkably long distances into the nutrient-free medium. Mycorhiza formation takes place almost immediately after the slowly advancing hyphae come in contact with the roots. The hyphae follow the roots into the agar far beyond the depth to which they would otherwise penetrate, and continue to form mycorhiza as they advance.

The type of infection that develops in the water-agar cultures appears to be identical with that in natural cranberry mycorhiza. After entering an epidermal cell (pl. 4, *B*), the hypha coils and branches freely within the lumen (pl. 4, *B* and *C*) until the enveloping mycelial mass finally fills the cell completely (pl. 5, *B*). The end result is digestion or disintegration of the mycelial mass within the cell exactly as it occurs in nature (*20, pp. 268–269*). The prevailing pattern of infection consists of infected epidermal cells, either single or in small groups, separated by larger areas of fungus-free tissue (pl. 5, *A* and *B*). Internal hyphae have been observed to pass from cell to cell (pl. 5, *C*), but apparently the plant inhibits an extensive spread of the fungus in this manner; the completely infected epidermal areas often encountered probably result from separate external infections. The fungus has never been observed to penetrate more deeply into the root than the epidermal layer. Infection is strictly limited to root tissue, never occurring above the root-stem transition zone, despite the fact that external mycelium frequently advances well up the stem under agar-culture conditions.



A, Sterile cranberry seed planted on modified McArdle agar February 5, 1934. *B*, Tube of same agar inoculated with cranberry root endophyte. *C* and *D*, Same cultures as in *A* and *B*, 29 days later. Note that seed culture remained sterile although medium is favorable to growth of endophyte. *E*, Average 16-weeks-old cranberry seedling grown on sterile distilled-water agar. × 1½.



A, Culture of cranberry seedlings on distilled-water agar, planted January 15, 1934, inoculated with the cranberry root endophyte January 29; photographed February 28. Plant *a* was removed March 10, when photomicrographs of mycorhiza, reproduced in plate 5, *A* and *B*, were made from its roots. × 1½. *B*, Cranberry mycorhizal fungus entering epidermal cells of cranberry seedling root in distilled-water agar culture; seed planted January 15, 1934, inoculated January 29; photographed March 13. × 963. *C*, Epidermal cell almost filled by mycorhizal mycelial complex. From same culture as *B*, photographed March 24, 1934. × 963.

Several questions immediately arise in regard to the significance of infections obtained in the manner described. Do they provide conclusive proof that the fungus is in fact identical with the endophyte that produces similar structures under natural conditions? In other words, is this true mycorhiza formation? Or is it more probable that the plants are partly starved when grown on nutrient-free agar and consequently have lost their ability to resist invasion by the fungus? Is the plant either injured or benefited as a result of the infection? Will fungi from other sources produce similar formations in the roots? Is *Phoma radicis* present systemically in both inoculated and uninoculated plants? If *Phoma*, rather than the fungus isolated by the writer, is the true endophyte, why does it not produce the "mycelial complexes" under these conditions as the cranberry fungus does? In an attempt to answer these and related questions numerous experiments have been conducted.

Agar rather than sterilized soil was used for the inoculation experiments because it appeared to offer certain advantages over soil, particularly in respect to the ease of varying the nutrient composition of the substrate and of observing the behavior of fungi, whether introduced intentionally or accidentally. The stock culture medium (p. 819) that had proved favorable to the fungus was first tried, with disappointing results. Roots coming in contact with the agar did not grow normally, but soon turned brown and failed to penetrate into the medium. (Compare pls. 3, *C*, and 4, *A*.) In the inoculated series the fungus enveloped the surface-lying roots with a dense weft of hyphae but only on rare occasions penetrated into epidermal cells to produce characteristic hyphal complexes. These few instances of infection, however, closely resembled mycorhiza and made it appear that the true endophyte was being used, but under conditions not conducive to abundant infection. Proceeding next on the assumption that the fungus might obtain food materials from roots if not available in the culture medium, nutrient-free agar was tried, resulting in immediate and at times extremely heavy infection as noted above.

RESULTS OF EXPERIMENTS

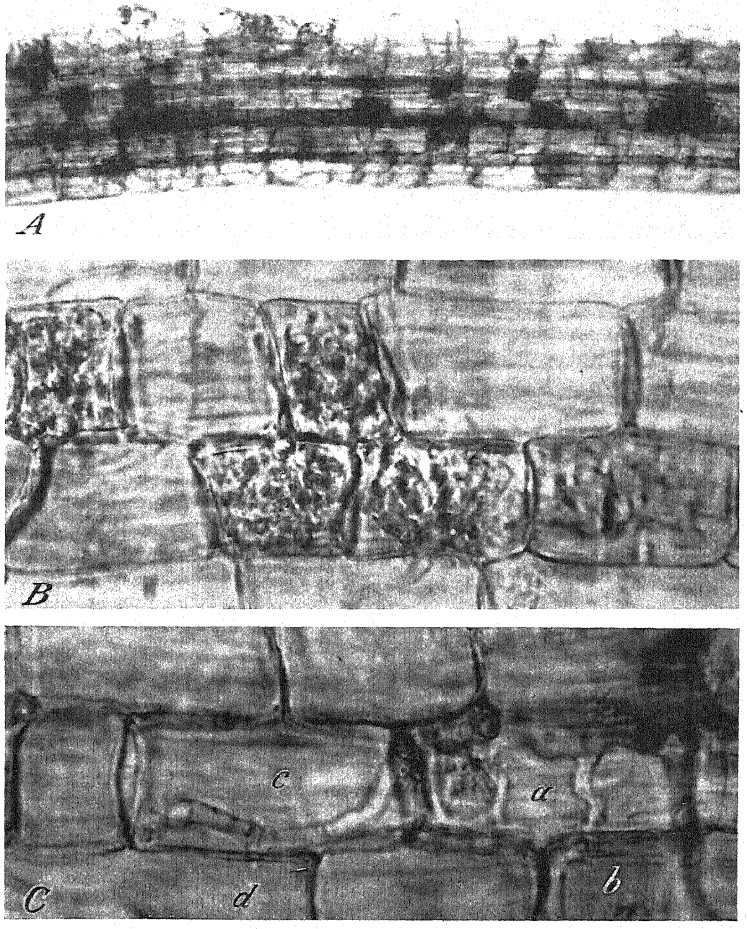
In a series of water-agar cultures started in the spring of 1935, 6 seeds were planted in each of 284 test tubes, 142 of which were inoculated with the cranberry endophyte. Frequent microscopic examination showed that plants in the inoculated series were invariably mycorhizal while those in the uninoculated checks lacked mycorhiza. At the end of 16 weeks 750 plants remained in the inoculated series and 767 in the checks. At this time the average height of 25 random plants in the inoculated series was 41 mm and in the uninoculated series it was 39 mm; both lots had an average of 7 true leaves. All the plants were then removed, with root systems intact, and were thoroughly air-dried and weighed. The weight per plant in the inoculated series was 3.64 mg and in the uninoculated series 3.63 mg. The seed reserve obviously must have supplied practically all mineral nutrients used by the plants grown under both conditions; the fungus did not seriously deplete the store; and nitrogen availability and starch formation must have been about equal in the two lots. It appears, then, that the fungus was neither injuriously parasitic nor beneficial to the plants under the conditions of the experiment.

Some curious results were obtained in another series of plantings in 1935, in which an attempt was made to find out whether the fungus derived certain substances or elements from the roots. With modified McArdle agar as a base, one after another of the chemical compounds was omitted, and by making substitutions in the formula, a single element at a time was eliminated. The formulas were made up as shown in table 1, with 15 g of agar and 1,000 cc of distilled water added to each except to the nutrient-free agar.

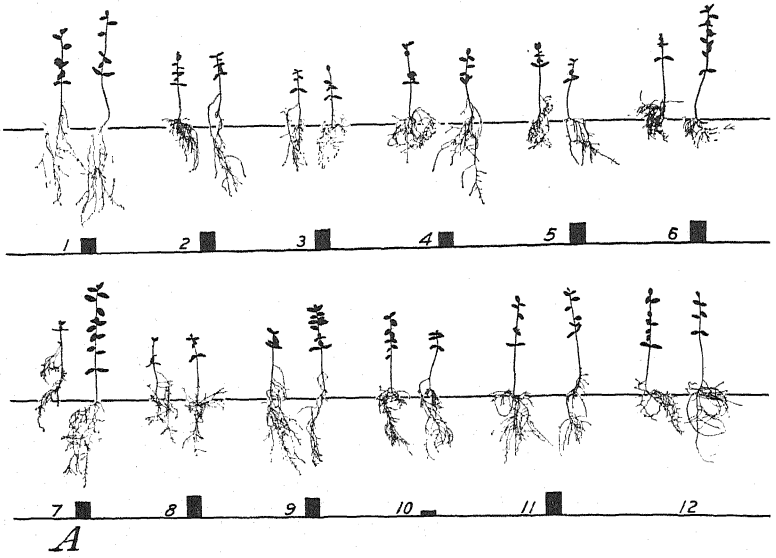
The cranberry fungus was grown for 2 months in a tube from each lot, after which transfers were made from the edges of the colonies to fresh lots of the same agars. The diameters of the colonies on the subcultures were measured when the colonies were 19 days old; the results are shown in plate 6, *A*. There was definite growth in every lot, though the sparse development of hyphae in No. 1 and the reduced rate of growth in No. 10 were decidedly subnormal. Growth was slightly subnormal in all others except Nos. 2 and 9.

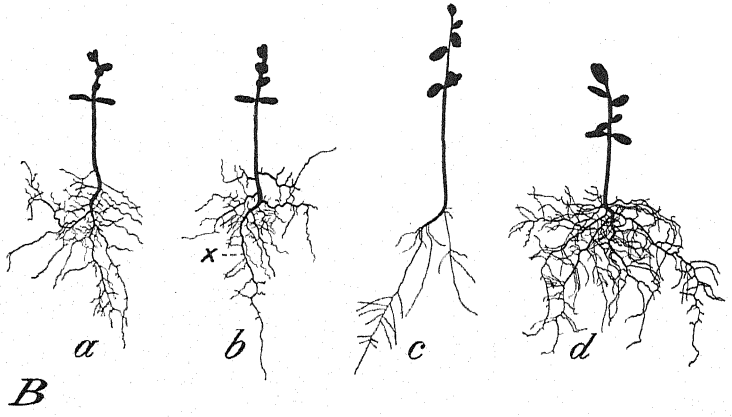
On February 5, seed from surface-sterilized berries were planted, five or six to the test tube, on the agars described above. Germination was irregular. Half of the tubes were inoculated with standard agar cultures of the cranberry fungus after the seed germinated. The other half were held as checks. The tubes were capped with waxed paper and kept in the greenhouse for 16 weeks. Roots from both inoculated and check lots were examined at frequent intervals for fungus infection. At the conclusion of the period 25 plants from each lot were measured, and all plants were carefully removed, air-dried for 4 months, and weighed.

Marked differences in growth were evident from the beginning, showing in such respects as height, color, and thickness of stems; size, number, and color of leaves; numbers and branching of roots; penetration of roots into the agar; and formation of adventitious roots that grew along the glass surface opposite the agar. Notes are summarized in table 2 and charted in plate 6, *A*.



A, Root from plant *a* (pl. 4, *A*), photographed March 10, 1934, showing distribution of infected (dark-colored) epidermal cells. × 167. *B*, Mycorhiza from plant *a* (pl. 4, *A*), photographed March 10, 1934; in four infected cells the mycelium is in early stages of digestion; in lower right the hyphal coils have not quite filled the cell. × 750. *C*, Early stages of infection in water-agar culture; hyphae branching and beginning to coil in cell *a* and passing through cell walls into *b* and *c*; a branch from *c* has started into *d*; photographed March 23, 1934. × 750.





A, Average plants in the 1935 agar-culture series (tables 1 and 2); in each pair, plant on left is from inoculated culture, on right from uninoculated culture; blocks represent relative diameters of cranberry mycorhizal fungus colonies on the respective agars (1–12) when 19 days old; individual plants were photographed and the prints were inked over after being assembled, thus making roots appear considerably heavier than they actually were. × approximately ½. *B*, Cranberry seedlings 60 days from date of planting, grown in (*a*) cork-sand mixture, not inoculated, not mycorhizal; (*b*) cork-sand mixture, inoculated with cranberry mycorhizal fungus, all roots above *x* infected; (*c*) distilled-water agar in test tube, inoculated with mycorhizal fungus, mycorhizal; (*d*) Wisconsin peat, mycorhizal; traced root by root under enlarging camera; diameter of roots enlarged for illustration. × Approximately ¾.

TABLE 1.—*Constituents*[1] *of media used for the agar-culture series of cranberry seedlings in 1935*

Lot No.[2]	Nutrient omitted	$MgSO_4$	$Ca(NO_3)_2$	KH_2PO_4	Carbohydrates			$MgCO_3$	$(NH_4)_2SO_4$	$CaCl_2$	$NH_4H_2PO_4$	K_2CO_3
					Maltose	Dextrose	Soluble starch					
		Gram	*Gram*	*Gram*	*Grams*	*Grams*	*Grams*	*Gram*	*Gram*	*Gram*	*Gram*	*Gram*
1	Carbohydrates	0.5	0.5	0.25								
2	$MgSO_4$		.5	.25	2.0	2.0						
3	$Ca(NO_3)_2$	.5		.25	2.0	2.0	2.0					
4	KH_2PO_4	.5	.5		2.0	2.0	2.0					
5	Sulphur		.5	.25	2.0	2.0	2.0	0.5				
6	Magnesium		.25	.25	2.0	2.0	2.0		0.25			
7	Calcium	.5		.25	2.0	2.0	2.0		.5			
8	Nitrogen	.5		.25	2.0	2.0	2.0			0.25		
9	Potassium	.5	.5		2.0	2.0	2.0				0.25	
10	Phosphorus	.5	.5		2.0	2.0	2.0					0.25
11		.5	.5	.25	2.0	2.0	2.0					

[1] Made up with 15 g of agar and 1,000 cc of distilled water.
[2] The check (lot No. 12, table 2), from which all nutrients were omitted, consisted of 1-percent distilled-water agar as described on p. 819.

TABLE 2.—*Data on agar-culture series of cranberry seedlings in 1935* [1]

Lot no. and description	Treatment	Plants	Height of stems	True leaves	Weight of plants	Characters of—		
						Stems	Leaves	Roots
		No.	*Mm*	*No.*	*Mg*			
1 (lacking carbohydrates).	Inoculated	191	42	9	4.1	Green	Large, green, vigorous	Mostly clear, long, slender, not stubby, penetrating agar readily, moderately branched. Mycorhiza formed freely in some plants, scarce or lacking in others.
	Not inoculated	210	43	8	3.8	do	do	Clear, long, slender, moderately branched, penetrating agar freely. No mycorhiza.
2 (lacking $MgSO_4$)	Inoculated [2]	215	29	7	4.8	Thick, reddish brown	Small, reddish	Light reddish brown, much branched, mostly penetrating agar about 10 mm, few individuals more deeply. Some mycorhizal cells in roots in agar.
	Not inoculated	214	29	6	3.9	Reddish brown	Medium size, dark green	Colored but not as brown as in inoculated plants; not stubby, rarely penetrating agar. No mycorhiza.
3 (lacking $Ca(NO_3)_2$)	Inoculated	187	27	4	4.2	Thick, reddish brown	Small, green	Light brown, moderately branched, mostly aerial or on glass, some penetration of agar, tendency toward stubbiness. No true mycorhiza, but fungus sometimes penetrated cells and occasionally formed a few coils. Both internal and external hyphae of huge diameter and gorged with oil.
	Not inoculated	182	25	5	3.7	Thick, dark red above cotyledons.	do	Red, short, moderately branched, penetrating agar slightly, not stubby. No mycorhiza.
4 (lacking KH_2PO_4)	Inoculated	213	38	7	4.7	Thick, red above cotyledon.	Medium size, green	Light reddish brown, very little penetration of agar, most growth and branching aerial or on glass, fairly long. Occasional mycorhizal cells formed.
	Not inoculated	204	30	7	3.8	Thick	Medium large, green	Light reddish brown, tendency to stubbiness, slight penetration of agar, roots mostly aerial or on glass, medium long. No mycorhiza.
5 (lacking S)	Inoculated	147	32	7	4.3	Red above and below cotyledons.	Small, newer ones with reddish cast.	Light reddish brown, profusely branched, stubby especially in contact with agar, short, very little penetration of agar. Occasionally a mycorhizal cell.
	Not inoculated	186	27	5	3.2		Pale, but general appearance of plants good.	Light reddish brown, comparatively long and slender, penetrating agar slightly, no stubbiness. No mycorhiza.

6 (lacking Mg)	Inoculated [3]	175	30	6	3.3	Thick	Very small, newer ones pale green.	Reddish brown, stubby to tuberculate in contact with agar, short, branching profusely, not penetrating agar. No mycorhiza, but fungus sometimes grew through collapsed epidermal cells.
	Not inoculated	180	48	10	4.5	Green, slender	Large, rich green	Almost clear, sparse, short, fine and cobwebby, moderately branched, not penetrating agar. No mycorhiza.
7 (lacking Ca)	Inoculated [3]	72	27	5	3.0	Red	Dwarfed, newer ones colorless to reddish green.	Reddish brown, very stubby, almost tuberculate in contact with agar, not penetrating, most branches extremely short. No mycorhiza.
	Not inoculated [4]	175	48	13	4.5	Green to reddish green	Large, dark green	Mostly clear, medium long, moderately branched, penetrating agar to some extent. No mycorhiza.
8 (lacking N)	Inoculated	201	28	4	4.4	Thick, straw-colored	Small, light green	Reddish brown, short, occasional slight penetration, stubby in contact with agar. Considerably branched. No mycorhiza but hyphae occasionally grew through collapsed epidermal cells. External hyphae large, gorged with oil.
	Not inoculated	198	28	5	3.7	Not so thick as in inoculated lot.	Small, deep green	Reddish brown, short, not stubby, moderately branched, penetrating agar only slightly. No mycorhiza.
9 (lacking K)	Inoculated	187	33	6	3.5		Small, newer ones pale	Light brown, considerable variation in growth and branching, not penetrating agar, sometimes tuberculate in contact with agar. No mycorhiza.
	Not inoculated	161	39	9	4.5	Mostly green, slender	Large, deep green, set close together. General appearance good.	Very light brown to almost clear, moderately long, moderately branched, not penetrating agar. No mycorhiza.
10 (lacking P)	Inoculated	188	37	7	4.4		Large, green	Light brown, medium long, somewhat stubby in and on agar, longer on glass, moderately to profusely branched. No mycorhiza.
	Not inoculated	210	27	5	2.9		Very small, yellowish red	Light brown, short, not penetrating agar, longer and moderately branched on glass, not stubby. No mycorhiza.

[1] Data in columns 4 to 6 are averages based on number of plants (column 3).
[2] Several plants failed to emerge from seed coats.
[3] Seed germinated poorly.
[4] Size and color of plants approached those of soil-grown plants of same age.

TABLE 2.—*Data on agar-culture series of cranberry seedlings in 1935*—Continued

Lot no. and description	Treatment	Plants	Height of stems	True leaves	Weight of plants	Characters of—		
						Stems	Leaves	Roots
		No.	*Mm*	*No.*	*Mg*			
11 (complete agar)	Inoculated	170	43	8	4.8	Red above cotyledons	Medium size, green	Light reddish brown, short and stubby in contact with agar, long and profusely branched on glass, some penetration of agar except in areas occupied by fungus colony. Occasional mycorhizal cells, hyphae sometimes growing through epidermal cells.
	Not inoculated	140	41	8	4.0	Not so red as in inoculated lot.	do	Light brown, stubby in contact with agar, penetrating slightly; long, slender, and moderately branched along glass. No mycorhiza.
12 (distilled water agar)	Inoculated	750	41	7	3.6	Reddish, slender	do	Rather short, not profusely branched, slender, many reddish, others mostly clear, penetrating agar freely and deeply. Mycorhiza formation invariable, infection at times extremely heavy.
	Not inoculated	767	39	7	3.6	Brownish green, slender	do	Long, slender, sparingly branched, light brown to clear or occasionally red, mostly in agar. No mycorhiza.

Any attempt to interpret these results evidently must be made by comparing the behavior of plants in nutrient-free agar (No. 12) with that of plants in either complete agar (No. 11) or in the agar in which the plants most nearly approximated normal soil growth (No. 7, uninoculated). The growth differences between inoculated and uninoculated plants on nutrient-free agar, as pointed out, were not measurable. On complete agar the only apparent difference was an increase of 20 percent in weight of the inoculated plants over the checks, while these inoculated plants were but 33⅓ percent heavier than either inoculated or uninoculated plants grown in nutrient-free agar, and were only slightly taller and produced but one more leaf. The plants that grew the tallest stems and the greatest number and largest sized leaves (No. 7, uninoculated) weighed slightly less than those on the inoculated complete-nutrient agar. In the light of these relations it must be concluded that wherever growth was subnormal as compared with plants in the nutrient-free agar, subnormal growth was due to direct injury exerted by the unbalanced substrate and not to deficiencies in the agar. Roots of subnormal plants responded by failure to penetrate the agar freely (in extreme cases becoming tuberculate in contact with it), or by turning brown or red, or by developing adventitiously or branching freely out of contact with the agar. This condition was apparently comparable to the root toxicity found by Friesleben (*9*, *10*, *11*) in using fungus-free sterilized peat, and lends support to Knudson's suggestion (*14*) that the failure of Rayner's agar-culture *Calluna* plants to form roots was probably directly attributable to conditions existing in the substrate. The cranberry plant, by way of contrast, with its remarkable rooting capacity, was able to survive on these unfavorable media for long periods, although stems, leaves, and roots in contact with the agar often showed injury.

Considering the combined effect of media and fungus on the plants, it was found that in three instances (Nos. 4, 5, and 10) the inoculated plants grew better than their uninoculated checks, and in three cases (Nos. 6, 7, and 9) the reverse was true. Where the inoculated plants were superior, there was a trace of mycorhizal infection in two lots, none in the other (No. 10); height of plants and general appearance were inferior to the water-agar cultures, though dry weight per plant was greater, and roots were abnormal. In those lots in which the checks were superior the plants grew decidedly better than the water-agar cultures in two cases (Nos. 6 and 7) and at least equally well in the other (No. 9), and the roots were almost normal in appearance. The plants in No. 1 also produced normal roots both in inoculated and check cultures; in growth they were intermediate between the water-agar and complete-agar cultures; and mycorhiza formation in inoculated plants in this carbohydrate-lacking medium was more abundant than in any other medium except water agar. Taking all these relations into consideration, the following conclusions appear to be justified:

(1) The culture media as made up were injurious to the plants except in Nos. 1, 6, 7, 11, 12, and possibly 9.

(2) The injury was of a toxic nature and was not due to specific nutrient deficiencies.

(3) Presence of the fungus in the cultures tended either to reduce the toxicity (Nos. 4, 5, and 10) or to intensify it (Nos. 6, 7, and 9),

according to the changes in the medium brought about by metabolic activity of the fungus.

(4) Little information was gained regarding the role played by the fungus in the mycorhizal association. However, it was noted that in nutrient-free agar (No. 12) inoculated plants were invariably mycorhizal but no heavier than their nonmycorhizal, uninoculated checks; while in the partial or complete nutrient media Nos. 2, 3, 4, 5, 8, 10, and 11, plants from inoculated cultures gained in weight over corresponding check plants without developing mycorhiza to any extent.

(5) In order to enter the roots freely the fungus must be partially starved, or the roots must be growing satisfactorily in the medium, or both.

(6) Absence of carbohydrates in the medium favors infection.

(7) Cranberry roots are so sensitive to the medium in which they are growing that caution must be exercised in attributing root or plant response to a single factor of the complex plant-medium-fungus association.

Similar changes in root and plant responses are brought about by the cranberry fungus in culture media prepared from extracts of plant substances. For example, corn-meal agar was detrimental to the growth of cranberry seedlings. The plants remained small, leaves were yellowish green, and the roots stopped growing abruptly where they touched the agar, but produced dense mats of reddish-brown branches along the sides of the tubes away from the agar. In the inoculated series plants were nearly twice as tall, leaves were larger and greener, and root development took place almost entirely within the agar. In malt-extract agar roots were incapable of penetrating the uninoculated medium and invariably produced jet-black tubercular or nodular structures where they touched it. Inoculated plants lacked both the blackening and tuberculariza tion, though roots did not penetrate into the agar.

In another series of experiments the ground-cork and sand mixture described by McArdle (*15, footnote p. 301*) was used as a medium instead of agar. Test tubes were filled to a depth of 6 cm, a small amount of the nutrient solution recommended by McArdle was added, and the tubes were autoclaved and inoculated with the cranberry fungus on December 1, 1932. By February 4, 1933, mycelium had penetrated about 1 cm in the tubes. Single sterile plants, which had been planted on standard agar (p. 819) 4 weeks previously as a test for sterility, were then transferred to a number of the tubes. Typical hyphal complex infection was present in roots when the first examination was made on March 14, and before the end of the month most of the roots were heavily infected. Root elongation soon ceased, and the tips enlarged and turned brown. Most of the tubes had dried out when the experiment was concluded April 12. In those tubes that were still moist, the roots had penetrated only 1 cm and lateral roots were short and stubby; but numerous new branches were breaking through just behind the arrested tips, some of which, not more than 3 mm long, were already mycorhizal. Root growth of plants in the uninoculated test tubes was entirely similar, except that in no instance was hyphal complex infection present. It was evident that in both lots some condition of aeration or chemical composition was not entirely favorable to root growth.

Root growth comparable in type and extent to that occurring in normal cranberry soil (peat) was finally attained, but not under pure-culture conditions. On March 3, 1933, ten 2-inch pots were partly filled with the cork-sand mixture and sterilized. Then, without attempting to maintain sterility further, inoculated cork-sand mixture from the December 1 cultures was added, and 10 seeds, transferred directly from surface-sterilized berries, were planted in each pot. Ten similar uninoculated pots were prepared. The two lots were placed some distance apart on a bed of coarse cinders on a greenhouse bench, sprinkled with tap water, and cared for in the routine manner, except that after germination a liberal application of nutrient solution (p. 828) was given every tenth day. Seed germinated and grew similarly in the two lots. On May 2, 12 inoculated and 6 check plants were removed and carefully washed, after which the root systems were accurately traced under an enlarging camera. All roots of the 18 plants were then examined under the microscope for mycorhizal infection.

Mycorhiza formed in all 12 inoculated plants. Practically all roots were infected throughout except near the growing points and occasionally deep in the pots, the general distribution and abundance of mycorhizal cells being very similar to naturally infected roots growing in peat. The roots of the check plants were entirely free from hyphal complexes, though external hyphae of contaminating fungi frequently grew along and around them. A careful comparison of the root systems failed to disclose any consistent differences in amount or type of branching or extent of development between inoculated and check plants. The roots of both lots were bright and clear throughout, exhibiting no symptoms of repression such as had developed when plants were grown in test tubes. A typical inoculated plant is illustrated in plate 6, *B*, *b*; an uninoculated plant grown in cork-sand mixture in *B*, *a*; and, for comparison, an inoculated plant grown on distilled water agar in *B*, *c*, and a normal peat-grown plant of the same age in *B*, *d*.

The final answer to the question whether mycorhiza formation is indispensable to the host will not be known until plants have been carried to the fruiting stage in an aseptic environment in the presence and in the absence of endophytes. This has not been accomplished with the cranberry or with any other species of the Ericaceae. It is submitted that the evidence presented definitely establishes the fungus used in the experiments as the one which produced hyphal complexes in roots from which it was isolated. Given the true endophyte, the next step toward solution of the problem will be to find a sterilizable medium in which both roots and fungus will grow normally under aseptic conditions. The extreme sensitivity of cranberry roots to their environment was conspicuous throughout the experiments just described. Friesleben (*9*, *10*, *11*) has shown that roots of numerous other heath plants will not develop normally in heat-sterilized peat soils.

SYNTHESIS EXPERIMENTS WITH THE ENDOPHYTES OF CHAMAEDAPHNE, LEDUM, AND VACCINIUM CANADENSE

In several of the agar-culture experiments reported above, parallel series of cranberry seedlings were inoculated with the endophytes of

Chamaedaphne, *Ledum*, and *Vaccinium canadense*. It was found that the three fungi were capable of entering epidermal root cells almost as readily as the cranberry fungus and that they produced similar hyphal complex structures, distributed along the roots in a similar manner, without appearing to be injuriously parasitic to the plants. Except in water agar, striking changes in plant and root growth were likewise brought about by inoculating the medium, although the four fungi did not always induce similar responses in the same medium.

Minor differences, correlated in large part with mycelial characteristics, were discernible in the hyphal complexes. For example, the *Vaccinium canadense* complex was always somewhat dark in gross appearance, owing to the color in hyphal walls (pl. 7), and the *Chamaedaphne* (pl. 8) and *Ledum* (pl. 9) complexes were more hyaline than those of the cranberry and *V. canadense* fungi because of thinner hyphal walls. Nevertheless, observed separately, any one of the three would readily pass for typical mycorhizal infection (pl. 10). There appears to be no reason to doubt that in nature these three fungi, and probably others as well, if present in the soil, could enter into mycorhizal union with the cranberry.

Friesleben (*9*, *10*, *11*) reported results similar to these in his experiments, but Rayner and Smith (*24*, *p. 262*) found a high degree of specificity with *Phoma radicis* strains.

CONCERNING PHOMA RADICIS

Rayner's hypothesis of mycorhizal relations in the heath family has held so conspicuous a place in the literature that her views should be given full consideration in discussing the subject. Briefly summed up, her hypothesis, with its implications, maintains that:

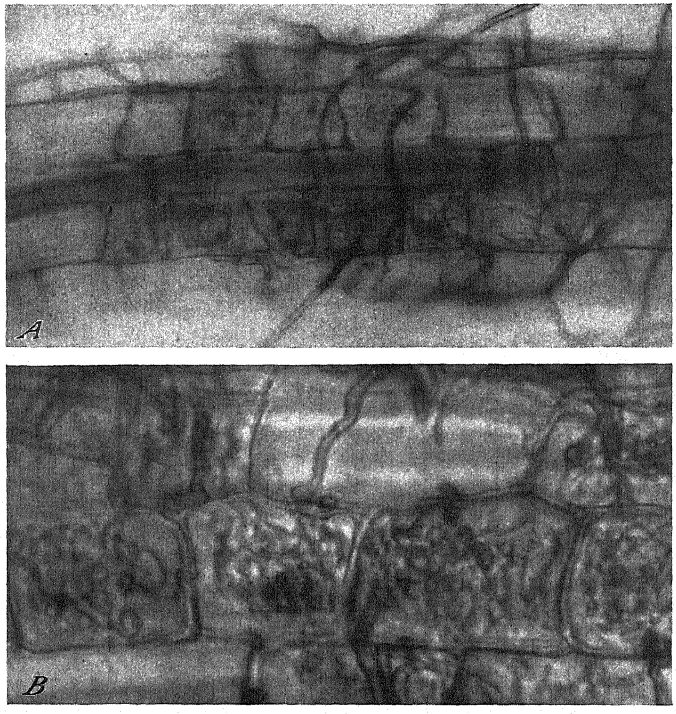
(1) The mycorhizal fungus of the heath family is *Phoma radicis*, a fungus which has specific strains closely restricted to the various members of the family.

(2) This *Phoma* ramifies through the entire plant organism—roots, stems, leaves, flowers, fruits, and seeds. In *Calluna* infection of the seed is limited to the coat, while in *Vaccinium* the mycelium advances into the endosperm.

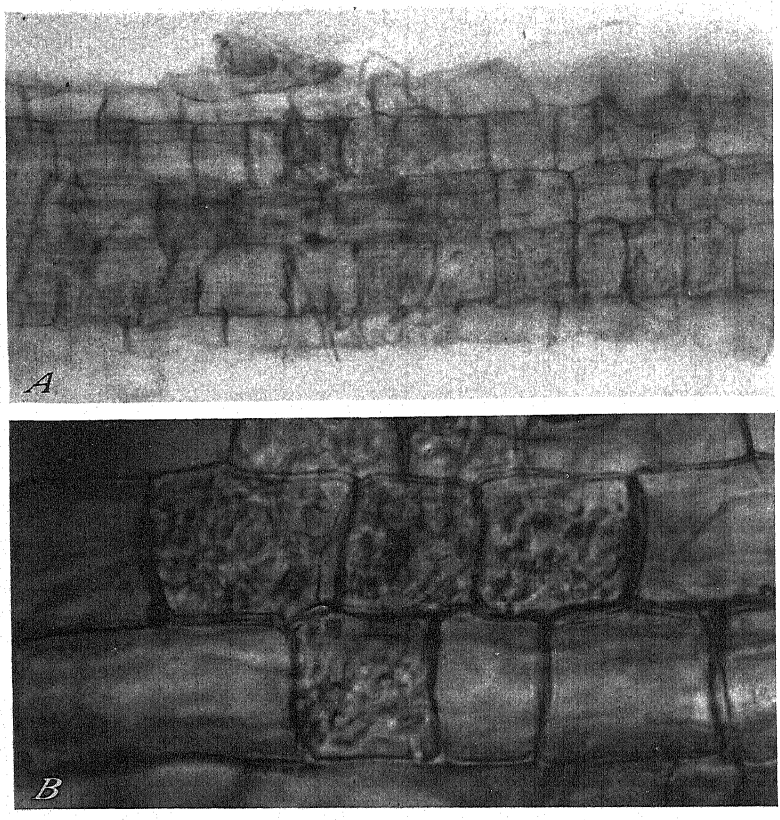
(3) Heath plants therefore cannot escape being infected in nature.

(4) In endosperm-infected species it is impossible to secure plants free from infection by any of the ordinary methods of seed sterilization (*21*, *p. 63*; *22*, *p. 381*).

(5) The mycelium exists in two dissimilar forms in the plant: (*a*) Normal-sized hyphae, easily visible under the microscope, develop intracellularly in roots (hyphal complexes) under suitable environmental conditions; they may arise from the other form of mycelium present in the root, but more often they represent new infections from the soil. (*b*) The hyphae in the systemic form of infection, in roots as well as in above-ground organs, are generally so attenuated in size that a specialized technique is required to make them visible. They form "a continuous network of mycelium in the middle lamellae of walls" (*20*, *p. 282*) and are "closely associated with the cell walls, pass[es] freely in and out of the cells" (*21*, *p. 62*). They sometimes occur in relatively large strands in intercellular spaces of leaves and stems (*17*, *p. 116 et seq.*).



Synthetic mycorhiza in water-agar culture of cranberry, produced by *Vaccinium canadense* endophyte: *A*, Distribution of infected cells, × 375; *B*, detailed structure, × 750.



Synthetic mycorhiza in water-agar culture of cranberry, produced by *Chamaedaphne* endophyte: *A*, Distribution of infected cells, × 375; *B*, detailed structure, × 750.

(6) Seedlings will not produce roots unless infected with *Phoma;* root formation per se is proof of infection. In this sense the mycorhizal relation is obligate to the host.

Each of these points will now be examined critically.

(1) Is *Phoma radicis* the mycorhizal fungus of all heath plants? In the author's experiments, four fungi, microscopically observed to originate from intracellular hyphal complex hyphae in roots of four heath species, reproduced the same type of infection when inoculated into roots of plants grown under completely controlled conditions. The four fungi entered cranberry roots with almost equal facility, and the plants were not injured by their presence so far as could be determined, i. e., there was no evidence of ordinary parasitism. Cultural characters proved conclusively that the four fungi were distinct from one another and from *Phoma radicis*. Christoph (*6*), Doak (*7*), and Friesleben (*9, 10, 11*) reported similar results with other ericaceous species. If the term "mycorhiza" is to be applied to the hyphal complex union of fungus and roots in the heath family, *Phoma radicis* is not the fungus involved in the cases cited, nor is the ability of one fungus species to form mycorhiza restricted to a single host species.

(2) The question of systemic infection by mycorhizal fungi in the heaths has been a matter of controversy, as is indicated in the review of literature. In the present investigations systemic infection in either the check or inoculated plants could not be verified despite thorough search in both fresh and fixed material handled as nearly as possible according to Rayner's published methods. Examination of a large assortment of prepared slides from healthy stems, buds, flowers, and young berries made in connection with a histological study of the cranberry false blossom disease also failed to disclose a single authentic instance of infection by "fine hyphae." Christoph (*6*), Knudson (*14*), and Friesleben (*10*) were likewise unable to find evidence of this type of infection in their material and held that the objects which Rayner called mycelium were most probably artifacts resulting from fixation or staining; but Rayner (*18, 22*) maintained that the inadequate technique of Christoph and Knudson prevented detection of the mycelium. She has not as yet commented on Friesleben's findings.

Some of the writer's material was submitted for examination to Dr. Ruth M. Addoms, who has conducted investigations on cranberry mycorhiza (*1, 2*), and her report is here quoted in part:

> Following are my notes on your cranberry cultures, which I have examined with some care:
>
> (A1) [Water-agar cultures inoculated with the cranberry fungus].—Roots long and red; fungus present externally and in epidermal cells.
>
> (A2) [Water-agar check for above, not inoculated].—No infection; not red.
>
> (B1) [Water-agar cultures inoculated with cranberry fungus, younger culture than above].—Roots red, much branched, infected.
>
> (B2, C2, and D2) [Uninoculated cultures on water agar, serving as checks for B1, C1, and D1].—Roots poorly developed, stems long; no infection.
>
> (C1) [Water-agar cultures inoculated with *Vaccinium canadense* fungus].—Roots not very long and blackish, heavily infected; fungus much coarser and browner than cranberry fungus, with longer cells; some fungus on surface of stem.
>
> (D1) [Water-agar cultures inoculated with *Ledum* fungus].—Roots not very long; definitely infected.
>
> As these notes show, I have not found infection in your check plants. Also I have not found the fungus within the cells of the stem, although this part of my examination has been less thorough than my study of the roots.

Evidence of a different sort, though somewhat indirect, may be cited. At different times Rayner has reported isolating *Phoma radicis* from immature fruits as well as from seeds of *Calluna;* in fact, judging from a careful perusal of her publications, her isolations have invariably come from above-ground plant parts; but when working with the cranberry (*21*), to her surprise she failed to find the fungus in seed cultures, a result, incidentally, confirmed by the writer (p. 819). The United States Department of Agriculture has conducted investigations of cranberry diseases continuously for 30 years, in the course of which cultures have been made from more than 100,000 individual berries, including a considerable number that were sound and immature, as reported by Shear (*28, 29, 30*); Shear, Stevens, and Bain (*31*); Rudolph and Franklin (*27*); Stevens (*33*); Stevens and Bain (*34, 35, 36*); Bain (*3*); and Bergman and Wilcox (*5*). *Phoma radicis* has not appeared once in all these cultures, a circumstance that appears incredible if the fungus is invariably present in the fruit tissues and grows as readily on ordinary culture media as Rayner's publications indicate.

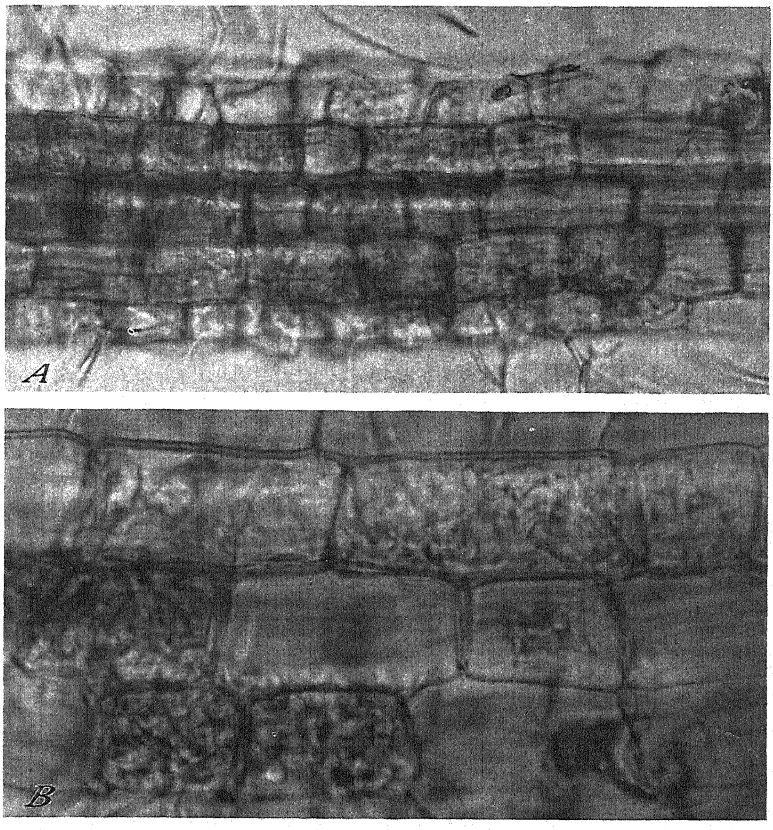
It appears to the writer that the weight of evidence is distinctly against either *Phoma radicis* or true mycorhizal fungi occurring systemically in the cranberry.

(3) Universal infection would be inevitable only in case systemic infection were invariable. In the writer's opinion, heath plants in general are mycorhizal because humus-inhabiting fungi of different species, capable of entering into the relation, are widely disseminated.

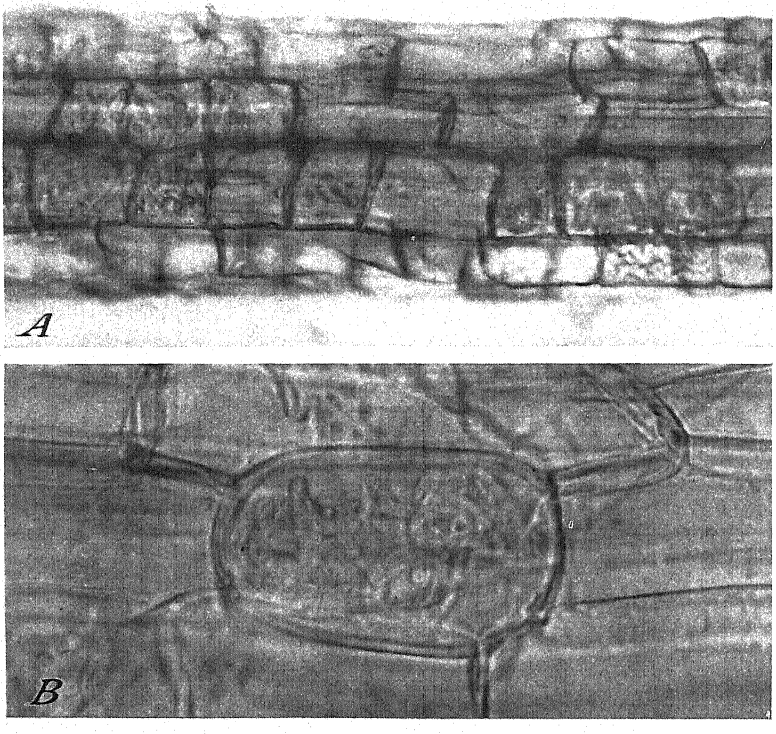
(4) The assumed impossibility of growing seedlings free from systemic infection is also based on universal invasion of seeds. However, mycorhizal infection, applying the term in its ordinary sense to the union of fungus and root (the hyphal complex relation in the case of heath plants), is quite another matter, and heath plants may be grown entirely free from the fungus or fungi involved, as has been demonstrated by the writer and by Stahl (*32*), Christoph (*6*), Knudson (*13, 14*), and Friesleben (*9, 10, 11*).

(5) No proof has yet been advanced that *Phoma radicis* assumes two different forms in different parts of the plant. The intracellular hyphal complex structures in roots, which Rayner attributes to *Phoma*, are produced by other fungi in *Calluna* (*6*), *Vaccinium corymbosum* and *V. pennsylvanicum* (*7*), *V. myrtillus*, *V. vitis-idaea*, and *V. uliginosum* (*10*), and *V. macrocarpon*.

(6) The hypothesis of obligate relationship in the heaths was advanced to explain certain definite results obtained in a series of experiments, and its validity depends upon how completely and exclusively it meets observed conditions in its own and related fields. In the experiments on which it was founded (*17*) plants grown in agar cultures from "adequately" sterilized seed of *Calluna* failed to produce roots until inoculated with a fungus derived from the fruit (or seed?) of the plant. Examination of the plants disclosed systemic infection by extremely attenuated hyphae in the inoculated series but no infection in the other lot. The fungus was therefore considered to be the stimulative factor bringing about root formation. To further strengthen the hypothesis, numerous cases of natural systemic infection in the same and related plants have been described subsequently, and in the genus *Vaccinium* (*21*) a similar series of agar plantings was made. Plants from seed sterilized in a manner which would have prevented root formation in *Calluna* here produced roots; but systemic infection



Synthetic mycorhiza in water-agar culture of cranberry, produced by *Ledum* endophyte: *A*, Distribution of infected cells, × 375; *B*, detailed structure, × 750.



Natural mycorhiza in cranberry: *A*, Distribution of infected cells, × 375; *B*, detailed structure, × 1,250.

was also present, the fungus having come from observed mycelium of the same type in the endosperm of resting seed. The coincidental development of roots with infection in these plants was cited as corroborative evidence of the obligate relationship.

Opposing this interpretation, Knudson (*13*, *14*) and Friesleben (*9*, *10*, *11*) attributed root suppression in sterilized media directly to an inhibitory factor existing in the substratum. In justification of their stand they have shown that if a given lot of sterilized seed is planted on two substrates differing in specified chemical or biochemical make-up, root growth is suppressed on one medium but not on the other. Such results would not be expected according to Rayner's hypothesis of root stimulation, because (1) if the sterilization were severe enough to completely eliminate seed-inhabiting fungi, roots should not develop in either lot, while (2) if the mycelium were not eliminated, both lots should produce roots. In the writer's opinion, the evidence for the cranberry is less convincing in this respect because the rooting capacity of the cranberry is too great to permit complete root suppression as long as any part of the stem is alive; but the roots are nevertheless extremely sensitive to the composition of the substratum.

The other phase of the obligate hypothesis, namely, actual existence of systemic infection, has been discussed above. Until the invariable existence of systemic infection is more conclusively demonstrated, and until there is more definite proof that root suppression is due solely to lack of such infection, the hypothesis of obligate root stimulation by fungi in the heath family cannot be considered valid.

SUMMARY

Mycorhizal fungi were isolated from "hyphal complex" cells in roots of four ericaceous species—*Vaccinium macrocarpon*, *V. canadense*, *Chamaedaphne calyculata*, and *Ledum groenlandicum*—a distinct type from each species.

The four fungi have not fruited in culture and hence could not be identified. They were obviously specifically distinct from each other and from *Phoma radicis*.

All four fungi produced the hyphal complex form of mycorhiza in cranberry seedlings grown in sterilized agar. The cranberry fungus also produced mycorhiza in cranberry seedlings when the latter were grown in an inoculated artificial soil composed of ground cork and sand. The hyphal complex form of mycorhiza was completely lacking when the plant cultures were not inoculated with the endophytes.

There was no evidence of injury due to parasitism after the seedlings were inoculated with the fungi.

In agar cultures the cranberry fungus entered into the mycorhizal relation much more freely if carbohydrates were withheld from the medium.

Seedlings grown in nutrient-free distilled-water agar derived no measurable benefit from the presence of mycorhiza.

Cranberry roots were extremely sensitive to the composition of the substrate. Their growth was more or less suppressed on nearly all nutrient agars tried.

Some nutrient agars were rendered still more toxic to roots when the endophytes were introduced into the media; others were made

decidedly less toxic. These changes in the media were not correlated with mycorhizal infection.

Systemic infection of the type attributed to *Phoma radicis* by some investigators could not be found either in the seedlings or in prepared slides from field-grown material.

The hypothesis of systemic infection by mycorhizal fungi and of its obligate relationship to root formation in the heath family is examined critically. It is shown that the hypothesis fails to conform to observed facts in some important respects.

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THE LOSS OF CAROTENE IN HAYS AND ALFALFA MEAL DURING STORAGE[1]

By EDWARD A. KANE, *assistant chemist*, HERBERT G. WISEMAN, *assistant chemist*, and C. A. CARY, *senior chemist, Division of Nutrition and Physiology, Bureau of Dairy Industry, United States Department of Agriculture*[2]

INTRODUCTION

For several years it has been recognized that the amount of carotene in plant materials is a measure of their vitamin A potency. This has been demonstrated best probably in the case of alfalfa hay (*7*).[3] It is also known that carotene is frequently deficient in farm rations (*6*, *14*, *15*, *16*). From an economic standpoint, therefore, it is important to know the conditions that influence the carotene content of farm feeds, especially of such feeds as alfalfa, timothy, and clover hays, which frequently furnish very nearly all the carotene in dairy rations, and which may, or may not, be rich sources of this factor (*20*).

This paper presents the results of experimental work conducted to determine the rate of loss of carotene in alfalfa and timothy hays and in alfalfa meals during storage under practical conditions at different seasons of the year. As the natural green color, which is used as a factor in the grading of market hays, is in general a most useful index of carotene content (*20*), the change in color that occurred during the storage of some of these hays was noted, and the results are included in this paper.

Considerable work has been done on the oxidative decomposition of pure carotene under various conditions. It is unnecessary to review this work here except to say that, although a number of oxidation products with vitamin A activity have been artificially prepared from carotene (*4*, *12*, *13*), the only naturally formed decomposition products that seem to have been tested for biological activity were tested in this laboratory and found to be inactive. There has, however, been work in other laboratories on the losses of carotene in the making and preserving of hays and meals by various methods; a review of this work may throw some light on the conditions that affect these losses.

REVIEW OF OTHER INVESTIGATIONS

Russell, Taylor, and Chichester (*19*) showed that carotene disappears rapidly from freshly cut plant materials. They observed a loss of 80 percent of the carotene in alfalfa during the first 24 hours of drying in the field. Similar losses have been noted by Hauge and Aitkenhead (*9*) and by Guilbert (*5*) and others when alfalfa was dried in the sun. Work with alfalfa, reported by this laboratory (*20*),

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[2] The work described in this paper was carried out in cooperation with the Bureau of Agricultural Economics, U. S. Department of Agriculture. Especial acknowledgment is made of the assistance of W. A. Wheeler, W. H. Hosterman, and W. L. Hall in the planning of these investigations and in preparing the meals; and of C. F. Welsh for the determinations of the color in both the hays and the meals.

[3] Reference is made by number (italic) to Literature Cited, p. 846.

shows that market hays frequently contain only one-tenth to one-fifth as much carotene as is ordinarily found in the growing plant.

A number of investigators have compared various methods of treating alfalfa to determine the conditions that affect the destruction of carotene. Russell (*18*), in his earlier work, found that leaves from alfalfa hay that had been quickly dried in a tunnel drier at 127° to 129° C., had more than seven times the vitamin A potency of leaves from a "poorly cured," "brownish green" hay that had been dried by exposure for several days in the field. In later experiments he and his coworkers (*19*) found that in two freshly cut samples of alfalfa dried either in a tunnel drier or in a rotary machine in which the material was momentarily exposed to a much higher temperature (650° to 750° C.), the carotene content per unit of dry matter was as great as that of freshly cut material, and 2 to 10 times as great as that of samples from the same hay that had been dried in the field. The loss of carotene in the field-dried hay was particularly rapid during daylight.

In the work of Hauge and Aitkenhead (*9*), and in later experiments by Hauge (*8*), it was found that, if freshly cut alfalfa was first autoclaved, there was no destruction of carotene when it was dried in the sun. Hauge and Aitkenhead also found that the autoclaved material did not lose carotene when incubated subsequently; whereas, when unautoclaved material was incubated, it lost carotene at a rate which increased as the temperature of incubation was increased from 4° to 37° C. They attributed the loss of carotene during the drying of alfalfa in sunlight to the action of enzymes the activity of which was increased by the higher temperatures produced by the sun's rays.

Guilbert (*5*) also found evidence of the presence of enzymes of this nature in alfalfa, but his results differ in several respects from those of Hauge and Aitkenhead. His analytical procedure, although it differed somewhat from theirs, would be expected to give similar results. Guilbert found that samples of alfalfa leaves that had been artificially dried by methods that should have destroyed any enzymes present, still lost carotene at a rapid rate when exposed to sunlight, and lost a considerable quantity even when standing in the dark. Alfalfa leaves that had been dried in vacuo at 100° C. lost 6.8 percent of their carotene when allowed to stand in the dark for 12 hours at 65°; and other leaves, similarly dried, lost nearly 10 times this amount when exposed to sunlight for the same length of time at the same temperature. Autoclaved samples of alfalfa lost 26.5 to 53.8 percent of their carotene when subsequently dried in sunlight, whereas unautoclaved samples that were directly dried in sunlight lost 46.6 to 69.5 percent.

In work with alfalfa meals, Guilbert also reports an artificially dehydrated sample which lost 83.3 percent of its carotene when exposed to sunlight for 40 hours. He compares the keeping qualities of the carotene in meals made from sun-dried and artificially-dried samples from each of three lots of alfalfa. In one lot the method of drying had no effect; in the other two, the losses of carotene were 50 percent greater in the artificially dried samples. The losses of carotene in these meals varied with the temperature at which they were stored. In a meal stored at 0° to 5° C., no loss was detectable in 8 weeks; in one stored at 20° to 30° about 30 percent was lost in that

time. Guilbert also determined the loss of carotene with three sun-dried samples of hay that were stored in the dark. They lost 9 percent of their carotene from November to April, and 30 percent from April to August.

The results recorded in the present paper have been presented in part before various groups, and abstracts of these reports have been published (*10*, *20*, *24*). In connection with various feeding experiments in this laboratory, a considerable number of market hays have been analyzed for carotene. Whenever in a lot, or purchase, of hay a bale has been found in which the hay showed evidence of fermentation such as a "caked" or "set" condition, or a "ground," "tobacco," or "sweated" odor, the carotene content has been decidedly lower than in bales in which this condition did not exist (*20*). As is well known, this spoilage, which is the result of bacterial action, is due to excessive moisture in the hay during storage. Woodward has studied this problem (*25*).

The fineness to which a hay is ground may also affect the rate of loss of carotene both in the preparation of meals and during subsequent storage. In grinding hays to a fine powder in a ball mill, a considerable part (average about 25 percent) of the carotene is lost, and the powdered material loses carotene very rapidly during subsequent storage (*23*). This does not occur, however, when the hays are ground in a Wiley mill equipped with a sieve with holes 0.5 mm in diameter.

From the foregoing statements it is evident that the destruction of carotene may proceed in hays in which all enzymes presumably have been destroyed. Loss of carotene occurs even when these hays are in the dark, and is accelerated greatly by exposure to sunlight, by an increase in the temperature at which they are allowed to stand, and by conditions favoring bacterial action in the hay. In considering the factors that influence the rate of destruction of carotene in hays, it must be borne in mind that pure carotene itself is oxidized on standing in air (*22*), that the process is much more rapid in sunlight, at higher temperatures, and generally when impurities are present (*2*, *3*). Evidence exists that the destruction of carotene in such preparations may be autocatalytic (*22*), or may be influenced by a number of catalysts (*3*) and anticatalysts or antioxidants (*1*, *3*, *21*). The process in hays, therefore, would be expected to be quite complicated and possibly quite variable.

EXPERIMENTS IN STORAGE OF HAYS

The hays used in this experiment were stored in bales in a dark, unheated barn loft at Beltsville, Md., and analyzed from time to time for carotene according to the usual procedure in this laboratory (*17*, *pp. 461–462*). The maximum and minimum outside daily temperatures were read and their average was taken as the "mean daily temperature." These mean daily temperatures were averaged to obtain the mean temperature for the periods of storage. The rate of loss of carotene in percentage per month was calculated by means of the equation $A(100-x)m=B$, in which x represents the rate, A and B are the initial and final carotene figures, respectively, for the period of storage, and m is the length of the period in months.

LOSS OF CAROTENE DURING STORAGE AT VARIOUS TEMPERATURES

ALFALFA HAY

The results of the experiment with alfalfa hay are shown in table 1, in which the percentage rates of loss of carotene per month are arranged in three groups according to the mean temperature during the period of storage. These three groups include samples stored at the following mean temperatures: (A) At 7.2° C. or less, (B) at 7.2°+ to 18.9°, and (C) at above 18.9°. Group A represents the temperatures that prevail at Beltsville, Md., during December, January, February, and frequently in March and November; group C the temperatures during June, July, August, and to a considerable extent in September; and group B the usual temperatures during the fall and spring months.

TABLE 1.—*Carotene content of alfalfa hays at different times during storage, percentage loss of carotene per month, and effect of temperature on this loss*

Samples of hay [1] (laboratory designation)	Date of analysis	Carotene content	Mean temperature since preceding analysis	Loss of carotene per month at temperatures indicated [2]		
				A, 7.2° C. or less	B, 7.2°+ to 18.9° C.	C, above 18.9° C.
		Milligrams per kilogram	° *C.*	*Percent*	*Percent*	*Percent*
Alfalfa hay, U. S. No. 1:	Mar. 25, 1933	37.1				
	May 19, 1933	34.5	12.2		3.9	
Lot 50, bale 1	June 8, 1933	31.4	19.4			13.8
	July 21, 1933	22.5	20.6			20.8
	Aug. 19, 1933	16.0	24.4			29.8
	Nov. 16, 1933	121.0				
	July 25, 1934	52.1	9.4		8.0	
	Nov. 19, 1934	46.2	16.1		3.1	
T-barn, bales 101, 102	Apr. 8, 1935	[3] 33.4	3.9	6.7		
	June 18, 1935	29.8	15.6		4.8	
	Aug. 29, 1935	22.9	23.3			10.5
	Jan. 4, 1934	33.4				
	Feb. 14, 1934	32.2	−0.6	2.6		
	July 26, 1934	21.0	13.9		7.6	
C-barn, bale 110	Nov. 19, 1934	19.0	16.1		2.6	
	Apr. 6, 1935	16.3	3.9	3.3		
	June 15, 1935	13.2	14.4		8.8	
	Jan. 20, 1934	30.5				
	July 16, 1934	16.7	12.2		9.8	
	Nov. 20, 1934	13.9	16.7		4.3	
Lot 57, bales 111, 112	Apr. 9, 1935	[3] 12.5	3.9	2.3		
	June 14, 1935	[3] 10.6	15.0		7.3	
	Mar. 22, 1934	38.0				
	July 5, 1934	25.2	16.1		11.2	
Lot 58, bales 117, 118	Nov. 20, 1934	[4] 15.8	17.8		9.9	
	Mar. 29, 1935	16.0	3.9	−1.0		
	June 12, 1935	14.4	13.9		4.2	
	Aug. 27, 1934	28.2				
	Jan. 24, 1935	21.1	9.4		5.8	
Lot 59, bales 1, 2	Mar. 29, 1935	20.2	3.9	2.0		
	June 22, 1935	17.5	15.0		5.0	
	Aug. 30, 1935	13.0	23.3			12.5
	Oct. 3, 1934	37.8				
	Jan. 18, 1935	29.5	7.2	6.8		
Lot 59, bale 3	Mar. 29, 1935	27.2	3.9	3.4		
	June 13, 1935	25.5	13.9		2.5	
	Aug. 30, 1935	19.1	23.3			10.5

[1] Hays graded by representatives of the Division of Hay, Feed, and Seed, Bureau of Agricultural Economics. Each "lot" of hay is a separate purchase.

[2] Data are arranged in 3 groups according to the temperature ranges indicated and depending upon the mean temperature at which the sample was stored during the period since the preceding analysis. Average temperature for 1933–35 at Beltsville, Md.: For winter months (December, January, February), 0.9° C.; for cool fall and spring months, 8.3°; for warm fall and spring months, 12.2°; and for summer months (June, July, August), 22.4°.

[3] 1 bale during this period was stored in the attic of the laboratory. This attic was unheated, but probably was warmer than the barn loft during the winter.

[4] 1 of triplicate determinations omitted; probably some destruction of carotene in it.

TABLE 1.—*Carotene content of alfalfa hays at different times during storage, percentage loss of carotene per month, and effect of temperature on this loss*—Continued

Samples of hay (laboratory designation)	Date of analysis	Carotene content	Mean temperature since preceding analysis	Loss of carotene per month at temperatures indicated		
				A, 7.2° C. or less	B, 7.2°+ to 18.9° C.	C, above 18.9° C.
		Milligrams per kilogram	° *C.*	*Percent*	*Percent*	*Percent*
Alfalfa hay, U. S. No. 1—Continued.						
	Nov. 5, 1934	43.4				
	Feb. 13, 1935	38.5	2.8	[5] 3.6		
Lot 64, 10 bales [5]	Apr. 15, 1935	34.0	7.2	[5] 5.8		
	June 21, 1935	29.3	16.7		[5] 6.5	
	Sept. 9, 1935	17.2	23.3			18.1
	Jan. 14, 1935	41.3				
	Mar. 30, 1935	39.0	3.3	2.2		
H. O., bale 1	June 21, 1935	28.3	15.0		10.9	
	Aug. 30, 1935	16.5	23.3			20.9
	Jan. 15, 1935	81.8				
	Mar. 30, 1935	72.5	3.3	4.7		
H. O., bale 1a	June 21, 1935	54.8	15.0		9.6	
	Aug. 29, 1935	30.0	23.3			23.3
Alfalfa hay, U. S. No. 2:						
	Jan. 31, 1935	14.1				
H. O., bale 2	Apr. 5, 1935	14.6	5.6	−1.0		
	Jan. 31, 1935	12.4				
H. O., bale 2a	Mar. 30, 1935	12.7	5.6	−1.0		
	Feb. 2, 1934	11.4				
	July 26, 1934	7.6	12.2		6.8	
Lot 54, bales 114, 115	Nov. 20, 1934	5.4	16.1		8.6	
	Apr. 16, 1935	[3] 4.7	3.9	2.8		
Alfalfa hay, U. S. No. 3:						
	Aug. 28, 1934	7.0				
Lot 61, bales 1, 2	Apr. 19, 1935	5.3	7.8		3.5	
	Sept. 26, 1934	8.7				
Lot 61, bale 3	Apr. 5, 1935	9.5	6.1	−1.0		
Average				2.6	6.6	17.8

[3] 1 bale during this period was stored in the attic of the laboratory. This attic was unheated, but probably was warmer than the barn loft during the winter.
[5] Same 10 bales used throughout except on Nov. 5, 1934.

It will be noted that the rate of loss of carotene during storage varied greatly with the different hays in each group shown in table 1, and that the average percentage loss per month with each group varied with the temperature at which the hays were stored. The rate of change in carotene content per month with the hays in group A varied from an increase of 1 percent to a loss of 6.8 percent; in group B, the loss varied from 2.5 to 11.2 percent; and in group C, from 10.5 to 29.8 percent. The average losses of carotene per month in these hays were, respectively, for group A, 2.6 percent; for group B, 6.6 percent; and for group C, 17.8 percent. Of the hays in group C, the average rate of loss of carotene for those that were stored the first summer after cutting was 21.1 percent per month, and that for the few samples (T-barn, bales 101 and 102; lot 59, bales 1 and 2; lot 59, bale 3) in storage the second summer after cutting was 11.2 percent per month.

These data for the loss of carotene during the storage of hays in the summer months indicate that the rate of loss of carotene under conditions otherwise comparable may possibly decrease with the age of the hay; but the errors and variations in determining such data are too great to permit a clear demonstration of this fact. A loss of carotene of 17.4 percent between November 16 and November 30, 1933, was observed with the hay marked T-barn, bales 101 and 102. This suggests that carotene may be lost at a very rapid rate in sun-

cured hays for some time after they are dried and baled. In none of the bales used in this experiment was there any evidence of fermentation or spoilage. The differences in the rate of loss of carotene with different hays at the same average temperature may actually be due in part to differences in the keeping qualities of the hays, but it must be borne in mind that the figures are derived from differences in determinations that are themselves subject to considerable error, and that samples stored at a given average temperature actually were exposed at times to quite different conditions.

Timothy Hay

The data obtained on the loss of carotene in timothy hay during storage are shown in table 2. It will be noted that the rate of decomposition of carotene during the storage of timothy hay is about the same as with alfalfa, and is similarly affected by variations in temperature. In 11 lots of timothy hay stored at temperatures between 6.0°+ and 18° C. the average loss of carotene was 6.3 percent per month; in 24 samples of alfalfa hay similarly stored the loss was 6.6 percent per month.

TABLE 2.—*Carotene content of timothy hays at different times during storage, percentage loss of carotene per month, and effect of temperature on this loss*

Samples of hay (laboratory designation) and date of analysis [1]	Carotene content	Mean temperature since preceding analysis	Loss of carotene per month at temperatures [2] indicated [3]	
			7.2° C. or less	7.2°+ to 17.8° C.
	Milligrams per kilogram	° *C.*	*Percent*	*Percent*
Lot 43, bales 107, 108:				
Mar. 1, 1934	19.9			
July 16, 1934	13.9	15.0		7.7
Lot 44, bales 1, 2:				
Aug. 28, 1934	9.2			
Jan. 3, 1935	6.8	10.6		6.9
May 1, 1935	6.3	5.0	1.9	
Lot 44, bales 115, 116:				
June 4, 1934	8.6			
Feb. 14, 1935	6.2	12.8		3.8
May 2, 1935	5.2	8.3		6.5
Lot 46, bales, 1, 2, 3, 4, 5:				
Dec. 11, 1934	23.6			
May 11, 1935	18.8	5.6	4.4	
Dec. 2, 1935	11.7	17.8		6.8
Lot 46, bale 6:				
Dec. 11, 1934	18.4			
Jan. 25, 1935	18.4	2.2	.0	
Apr. 25, 1935	17.0	5.6	2.6	
Dec. 2, 1935	11.7	17.8		5.0
Lot 48, bales, 1, 2, 3, 4, 5:				
Jan. 18, 1935	10.7			
Apr. 18, 1935	9.6	4.4	3.5	
Dec. 4, 1935	7.0	17.2		4.0
Lot 48, bale 6:				
Jan. 17, 1935	10.3			
Apr. 18, 1935	6.7	4.4	13.2	
Dec. 5, 1935	6.4	17.2		.6
Lot 39, bales 101, 102:				
Jan. 6, 1934	9.2			
July 16, 1934	4.5	10.0		10.7
Lot 42, bales 104, 105:				
Jan. 6, 1934	9.0			
July 5, 1934	4.7	9.4		10.3
Average			4.3	6.2

[1] Each lot of hay is a separate purchase.

[2] Average temperature for 1933–35 at Beltsville, Md.: For winter months (December, January, February) 0.9° C.; for cool fall and spring months, 8.3°; for warm fall and spring months, 12.2°; and for summer months (June, July, August), 22.4°.

[3] Results arranged in 2 groups according to mean temperature during periods between analyses.

Clover Hay

One lot of clover hay was included in the experiment. On June 24, 1934, it contained 16.8 mg of carotene per kilogram of dry matter. On January 2, 1935, and April 15, 1935, the carotene determinations each gave 8.8 mg per kilogram. The average temperature for the total period of storage was 11.1° C., and the average percentage loss of carotene per month was 6.3 percent. In six lots of alfalfa that were stored at temperatures ranging from 9.4° to 12.8° C., the average loss of carotene per month was 6.6 percent.

Relation of Green Color in Hay to Carotene Content

The writers have compared the decrease in green color during storage, as determined by hay specialists of the Bureau of Agricultural Economics, with the loss of carotene during the storage of several lots of alfalfa hay. In one lot there was a decrease in green color from 71 percent to 56 percent—a loss of 21 percent of the natural color originally present. The corresponding loss in carotene content was from 32 to 13 mg per kilogram, or nearly 60 percent. With another hay a decrease in color from 69 to 54 percent—a loss of 22 percent—was accompanied by a drop in the carotene content from 38 to 14 mg per kilogram, or somewhat over 60 percent. A third lot of hay, which changed from 55 percent color to 44 percent—a loss of 20 percent—lost 70 percent of its carotene content. Apparently the percentage loss of carotene is much greater than the corresponding percentage loss of natural green color—about three times as great with the samples just cited.

Experiment in Storage of Meals

For the study of the loss of carotene during the storage of meals made from alfalfa hay, several bales were taken from each of four lots, or purchases, of hay. The bales selected from each lot were similar in color and leafiness. One bale from each lot was graded by hay specialists of the Bureau of Agricultural Economics, and different portions of it were ground by them in a hammer mill to different degrees of fineness, so that the particles passed through ⅛-, ¼-, or ¾-inch circular mesh screens during grinding.

The color and the carotene content of these meals were then determined, and the determinations were repeated from time to time during storage from about April 1, 1934, to October 1, 1935. Samples of the meals were stored in individual bags in a dark, unheated part of a building in Washington, D. C. This place was somewhat more sheltered from the effect of outdoor weather changes than the barn loft referred to above. The other bales from each lot of hay were stored in this loft and analyzed from time to time. The outdoor temperatures were read at Beltsville (near Washington, D. C.); and the average temperatures for the periods of storage of the meals and the rates of loss of carotene during storage were calculated in the same manner as for the hay experiment.

Determinations of Carotene Content and Color

The results of the analyses for the carotene content of the meals, as determined from time to time during storage, are given in table 3.

TABLE 3.—*Losses of carotene and green color during the storage of meals made from alfalfa hay*

Meal prepared from bale no.—	Carotene content					Retention of natural green color					Green color of the hay on Apr. 7, 1934 [1]
	Date of analysis	Meal ground to pass—			Average	Date observed	Meal ground to pass—			Average	
		⅛-inch mesh	¼-inch mesh	¾-inch mesh			⅛-inch mesh	¼-inch mesh	¾-inch mesh		
		Milligrams per kilogram	*Milligrams per kilogram*	*Milligrams per kilogram*	*Milligrams per kilogram*		*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*
116	Apr. 23, 1934	12.8	13.5	12.5	12.9	Apr. 11, 1934	35	33	30	33	57
	Dec. 15, 1934	8.2	8.8	7.5	8.2	Nov. 20, 1934	34	33	29	32	
	May 21, 1935	6.1	7.8	5.4	6.4	Apr. 5, 1935	28	26	25	26	
	Oct. 14, 1935	5.0	5.0	5.0	5.0	Oct. 2, 1935	32	26	20	26	
119	Apr. 25, 1934	43.9	39.2	38.1	40.4	Apr. 11, 1934	48	47	48	48	73
	Nov. 30, 1934	29.4	25.5	21.8	25.6	Nov. 20, 1934	39	39	33	37	
	May 22, 1935	20.4	19.0	[2] 18.3	19.2	Apr. 5, 1935	33	36	31	33	
	Sept. 4, 1935	14.3	14.8	12.6	13.9	Oct. 2, 1935	35	34	24	31	
109	Mar. 28 and Apr. 9, 1934	43.9	42.1	[3] 45.3	43.8	Apr. 11, 1934	60	54	53	56	71
	Nov. 24 and Nov. 30, 1934	24.5	24.4	23.1	24.0	Nov. 20, 1934	55	50	48	51	
	May 21, 1935	21.1	[2] 19.4	[2] 21.1	20.5	Apr. 5, 1935	43	40	41	41	
	Oct. 12, 1935	13.6	13.6	13.9	13.7	Oct. 2, 1935	45	41	39	42	
113	Mar. 28, 1934	24.0	27.2	26.6	25.9	Apr. 11, 1934	53	52	53	53	72
	Nov. 27, 1934	16.3	18.7	18.3	17.8	Nov. 20, 1934	42	42	42	42	
	June 5, 1935	12.3	15.4	14.5	14.1	Apr. 5, 1935	39	41	38	39	
	Sept. 5, 1935	[4] 9.9	10.1	9.0	9.7	Oct. 2, 1935	39	33	40	37	

[1] The percentage of natural green color in the hay is not comparable with that in the meals.
[2] Determined May 13.
[3] Determined Apr. 9 and Apr. 16.
[4] Determined Sept. 4.

Table 4 shows the total percentage loss of carotene for the whole period of storage for each sample of meal.

TABLE 4.—*Total percentage loss of carotene and green color in alfalfa meals during period of storage (about Apr. 1, 1934, to Oct. 1, 1935)*

Meal prepared from hay bale no. —	Loss of carotene in meal ground to pass—			Loss of green color in meals ground to pass—		
	⅛-inch mesh	¼-inch mesh	¾-inch mesh	⅛-inch mesh	¼-inch mesh	¾-inch mesh
	Percent	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
116	60.9	63.0	60.0	8.6	21.2	33.3
119	67.4	62.2	66.9	27.1	27.7	50.0
109	69.0	67.7	69.3	25.0	24.1	26.4
113	58.8	62.8	66.2	26.4	36.5	24.5
Average	64.0	63.9	65.6	21.8	27.3	33.5

In table 5 the rates of loss of carotene in the meals and those in the corresponding samples of baled hay are compared for different outdoor temperatures.

The percentage of natural green color retained in the meals is shown in table 3 along with the data for carotene content. The total percentage loss of green color as compared with that of carotene is shown in table 4.

TABLE 5.—*Comparison of the rates of loss of carotene in alfalfa meals and corresponding baled hays, stored at different average temperatures*

Meal prepared from bale no.—	Loss of carotene in the meals[1]						Loss of carotene in corresponding hays stored in bales			
	Average outdoor temperature	Per month	Average outdoor temperature	Per month	Average outdoor temperature	Per month	Average outdoor temperature	Per month	Average outdoor temperature	Per month
	° *C.*	*Percent*	° *C.*	*Percent*	° *C.*	*Percent*	° *C.*	*Percent*	° *C.*	*Percent*
116	6.1	4.6	16.7	5.7	20.6	5.0	3.9	2.8	16.1	8.6
119	5.6	4.1	17.8	6.3	22.2	8.7	3.9	[2] +1.0	15.6	7.4
109	5.6	2.7	17.2	7.6	20.6	7.9	3.9	3.3	15.0	5.4
113	7.2	3.6	17.2	4.6	22.8	11.9	3.9	2.3	15.6	5.8
Average	6.1	3.8	17.2	6.0	21.6	8.4	3.9	1.8	15.6	6.8

[1] Each datum under percent per month is the average for all grindings from each bale for the temperature indicated.
[2] With this hay there was an apparent gain of 1 percent per month instead of a loss during storage.

From the data in tables 3, 4, and 5, the following conclusions appear justified:

(1) The rate of loss of carotene in the meals was not affected by the fineness to which they were ground. For the total period of storage, the meals ground to pass ⅛-, ¼-, and ¾-inch meshes lost on an average 64.0, 63.9, and 65.6 percent, respectively.

(2) The percentage loss of color in the meals was unquestionably much smaller than that of carotene. The changes in color, however (table 4), are quite confusing. True, the meals faded during storage, and on an average the loss of green color was greater in the more coarsely ground meals, but the individual results are inconsistent.

(3) When due allowance is made for the errors in the data, it is evident that the rate of loss of carotene in the meals during storage was quite similar to that in the corresponding hays, and that in both cases these losses were similarly affected by the temperature at which the samples were stored.

In considering the loss of carotene in the meals, the question arises as to the loss that may have occurred during the grinding of the meals. A comparison of the results for the hays with those for the meals would have no bearing on this question because in making the determination for carotene, all hays and meals were finally ground to the same degree of fineness. The finest meals, however, were about six times as coarse as the material used for analysis, which was ground to pass through the finest mesh screen of the Wiley mill. Unpublished data indicate that grinding in this way does not destroy the carotene. It would seem, therefore, that the grinding of the meals stored in this experiment may likewise have been accomplished without a considerable loss of carotene.

SUMMARY

Data on the rate of loss of carotene and of color in hays and alfalfa meals during storage are presented.

It appears that on an average the rates of loss of carotene in alfalfa, timothy, and clover hays under comparable conditions are quite simi-

lar. Therefore, the results for the different hays, taken together, may be summarized as follows:

Twenty-two samples of baled hays that were stored in a rather dark, unheated barn loft when the outside temperature was 7.2° C. or less (as is the case in December, January, February, and frequently in March and November, at Beltsville, Md., lost on an average about 3 percent of their carotene content per month.

With 34 samples of hay, which were similarly stored when the average outdoor temperatures ranged from 7.2°+ to 18.9° C. (as in April, May, October, and possibly September, at Beltsville), the average loss of carotene was 6.5 percent per month.

At outside temperatures above 18.9° C., the loss of carotene during storage increased greatly. For six samples of hay that were stored during the first summer after cutting, the loss was 21 percent per month, and for three samples stored further for a second summer, it was about 11 percent per month.

Meals from alfalfa ground to pass ⅛-, ¼-, and ¾-inch mesh screens lost carotene at the same rate during storage regardless of the degree of fineness to which they were ground, and at practically the same rate as the corresponding baled samples of hay.

With hays and meals, stored as above described, the percentage rate of loss of carotene was much more rapid than that of their natural green color. This fact must be taken into consideration in judging the carotene content or vitamin A potency of a hay from its color.

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A STATISTICAL STUDY OF SAMPLING IN FIELD SURVEYS OF THE FALL POPULATION OF THE EUROPEAN CORN BORER[1]

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INTRODUCTION

Surveys of insect abundance constitute an important part of the activities of economic entomologists. Such surveys not only provide fundamental information about insect pests on which control activities may be based but also may be used in studying the ecological relationships of these pests under actual field conditions and may thus contribute to the development and improvement of control methods. Obviously the methods used in the surveys of different insects and for different purposes differ from one another, depending as they do on the biology of the insect species and the objectives of the surveys.

The qualitative biological aspects of insect surveys are usually given adequate consideration, but the quantitative aspects of the sampling variability of all units of area-wide surveys have seldom, if ever, been analyzed under actual field conditions. For the analysis of the quantitative aspects of the sampling variability of such area-wide surveys of insect populations, the methods of analysis of the variance developed and introduced by Fisher (*4*)[3] are perfectly adapted. In the present paper these methods are applied to a study of sampling variability in the fall survey of the number of larvae per 100 cornstalks in the area infested by the one-generation brood of the European corn borer (*Pyrausta nubilalis* Hbn.)

SURVEY METHODS AND DATA

Beginning with the year 1919, surveys to ascertain the abundance of the mature European corn borer larvae in the standing cornstalks in the one-generation area have been made each year by the Bureau of Entomology and Plant Quarantine, in cooperation with various State agencies. The data used in the present analysis cover 66 county units, part of which were surveyed in 1931 and part in 1932. For certain counties the data for both years, as separate units, were used, but the sets for the 2 years are not duplicates, some counties appearing 1 year but not the other. For both years counties located in Michigan, Ohio, Pennsylvania, and New York were included and the whole treated as one set of data. The detailed records are on file at the office of the United States Department of Agriculture laboratory for European corn borer research, Toledo, Ohio.

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[3] Reference is made by number (italic) to Literature Cited, p. 871.

As the infestation of feeding larvae in the one-generation area is confined entirely to the cornfields and almost entirely to the corn plants in the fields, the cornfield has constituted the basic unit of the surveys, and data were taken only with regard to the infestation in the corn plants.

The intensity of infestation was expressed as the number of borers per 100 plants and was estimated by determining the number of infested stalks out of 100 and the number of borers per infested stalk. The field was divided into quarters and from the approximate center of each quarter 25 consecutive plants were gathered. From the total number of infested stalks in the 25 stalk samples the average percentage of infested plants in the field or the number of infested plants per 100 was obtained. Then the infested stalks were examined in order to determine the average number of borers per stalk. In areas where the level of infestation in general was low, all the infested plants up to a maximum of 5 from the 100 plants were dissected. In areas where the level of infestation was higher, a maximum of 10 plants, if available, were dissected. The product of the number of infested plants in 100 and the average number of borers per infested plant gave the estimate of the number of borers per 100 plants in the field.

In conducting the surveys, a single county was almost invariably taken as the unit for which the sampling was carried out and the mean level of infestation reported. In general, the counties surveyed were fairly uniform in size and served very satisfactorily as basic areas for the analysis of the sampling variance. In the counties in which the infestation was expected to be relatively low, 25 fields were sampled, and in counties where the infestation was expected to be relatively higher, 20 fields were sampled. The fields were taken at random over the county and later combined into four groups, termed "sections", each group containing the same number of fields.[4]

The two main objectives kept in mind in the analysis of the data presented in this paper were (1) to determine the relative influence, on the reliability of the estimates of the field mean number of borers per 100 plants, of the variability (*a*) of the samples, taken in each field, from which the average percentage of infested stalks was computed and (*b*) of the samples from which the average number of borers per infested stalk was computed, and (2) to determine the amount of variability between fields within a county relevant to the reliability of the estimate of the county mean number of borers per 100 plants, and to compare it with the variability of samples within fields.

ANALYSIS OF THE SAMPLING VARIABILITY OF THE ESTIMATES OF THE FIELD MEANS

VARIANCE OF A PRODUCT DERIVED FROM THE VARIANCE OF THE FACTORS

As described, the sampling within fields was carried out by making two estimates in each field, one to determine the number of infested stalks per 100 and the other to determine the number of borers per infested stalk, the product of the two determinations giving an estimate of the average number of borers per 100 stalks. The chief problem in the analysis of the sampling within a field is, therefore, to assign to each factor the portion of the total sampling variability of the

[4] Where 25 fields were sampled, the thirteenth field was arbitrarily discarded.

estimate of the field mean which would be influenced by increasing or decreasing the number of samples in each set independently, and to express these portions in comparable terms.

This problem of analysis may be approached by the use of certain equations whereby the mean product, the variability of this product, and the variability of each factor of the product may be estimated. The mean product in terms of the constants of the factors may be estimated by the equation

$$\overline{X} = \overline{p}\,\overline{b} \qquad \text{Equation 1}$$

in which $\overline{X}$ is the estimated mean number of borers per 100 plants, $\overline{p}$ is the mean percentage of infestation, or the number of infested stalks per 100 stalks, and $\overline{b}$ is the mean number of borers per infested plant.

The variance of the product, also in terms of the constants of the factors, may be estimated by the equation

$$\sigma_{\bar{x}}^2 = \overline{p}^2\left(\frac{\sigma_b^2}{n'_b}\right) + \overline{b}^2\left(\frac{\sigma_p^2}{n'_p}\right) \qquad \text{Equation 2}$$

in which p is the percentage of infested stalks out of the 25 stalk samples, b is the number of borers in an infested stalk, n'_p and n'_b are, respectively, the number of infestation samples and the number of plants dissected, σ_p and σ_b are the standard deviations of the two factors, and σ_p^2 and σ_b^2 are the corresponding variances.

The variance of the percentage of infested stalks out of the 25 stalk samples may be estimated by the equation

$$\sigma_p^2 = \frac{\Sigma p^2 - \frac{(\Sigma p)^2}{n'_p}}{n_p} \qquad \text{Equation 3}$$

in which n_p is the number of degrees of freedom available for estimating σ_p^2, or $n'_p - 1$.

The variance of the number of borers in an infested plant may be estimated by the equation

$$\sigma_b^2 = \frac{\Sigma b^2 - \frac{(\Sigma b)^2}{n'_b}}{n_b} \qquad \text{Equation 4}$$

in which n_b is the number of degrees of freedom available for estimating σ_b^2, or $n'_b - 1$.

A Comparison of the Variances of a Product Obtained by Two Methods

The variance of the field mean number of borers per 100 plants is underestimated when equation 2 is used, if a correlation exists between the number of infested stalks in the 25 stalk samples and the mean number of borers per infested plant in the corresponding quarters of the fields. Owing to the way the data were recorded in the fields, the correlation within fields could not be calculated for the years 1931 and 1932 when the data for this paper were obtained. But from data obtained in 1935 from 100 fields in Lenawee County, Mich.,

and the counties of Fulton, Hancock, Henry, Lucas, Ottawa, Putnam, and Sandusky, Ohio, the fact of a correlation between the two factors within fields was established and the effect this had on the standard error of the field mean number of borers per 100 plants was determined.

To show the existence of a correlation between the two factors just referred to, the number of infested plants out of 25 and the mean number of borers per infested plant were tabulated for the quarter of each of the 100 fields having the largest number of infested plants. Then the mean of the 100 samples was calculated for each factor. Similarly the data were tabulated and averaged separately for each of the other three quarters of each field. The mean numbers of infested plants out of 25 were 13.09, 10.53, 8.69, and 6.02 for the four quarters, while the mean numbers of borers per infested plant were, respectively, 1.75, 1.68, 1.55, and 1.39 for the same quarters. These data show that a decrease in the mean number of borers per infested plant is associated with a decrease in the number of infested plants.

The effect of this correlation on the standard error of the field mean number of borers per 100 plants was determined by completing two sets of calculations for each of the fields and comparing the mean results for groups of fields in the following manner: The first set of calculations was made to determine the mean number of borers per 100 plants for each field by equation 1 and to estimate the variance of the mean by equation 2. For the second set of calculations the product for each quarter of the field was obtained by multiplying the number of infested plants out of 25 by the mean number of borers per infested plant (2 or 3 plants were dissected in each quarter) and multiplying the result by 4 to obtain the mean number of borers per 100 plants for each quarter. The mean number of borers per 100 plants for each field was then obtained by averaging the results for the quarters of the fields. The variance of this general mean was estimated by computing the squared deviations of the product for each quarter from the mean product for the field and dividing by 4. The fields were then arranged in 5 groups, each of 20 fields, according to the field mean number of borers per 100 plants obtained from the first set of calculations. The group mean numbers of borers per 100 plants and the group mean standard errors are given in table 1 for each of the two methods of calculation.

TABLE 1.—*Range of estimates of the field mean number of European corn borers per 100 plants and the group mean standard errors derived by two methods of calculation, from 100 fields in northwestern Ohio and southeastern Michigan, 1935*

First method		Second method	
Mean	Standard error	Mean	Standard error
Number	*Number*	*Number*	*Number*
15.1	5.25	15.7	5.63
32.5	9.65	33.2	10.41
60.0	18.09	61.5	18.03
81.1	24.36	84.1	25.04
151.2	41.21	156.9	43.21

It may be observed from table 1 that both the field mean number of borers per 100 plants of the groups of fields and the mean standard error are slightly underestimated by using equations 1 and 2, The average of the five groups shows that the standard error is underestimated about 3.7 percent. It appears, therefore, that on the average the standard error of the field mean number of borers per 100 plants may be estimated rather closely by using equations 1 and 2. On the basis of this comparison the validity of the results from the study described in this paper is assured if the 100 fields surveyed in 1935 may be taken as representative of the conditions existing in fields in general.

In order to assign to each of the two sets of samples taken in each field of the 1931 and 1932 surveys—one set to determine the number of infested stalks per 100 and the other to determine the number of borers per infested stalk—the portion of the total sampling variability of the estimates of the field mean numbers of borers per 100 plants which would be influenced by increasing or decreasing the number of samples in each set independently and to express these portions in comparable terms, the use of equations 1 and 2 is necessary. But these equations are incomplete because they make no allowance for any correlation which might exist between the two sets of samples. The complete form of the equations has been given by Reed (7).

The complete form of equation 1, adapted from Reed, is obtained by adding the term $r\sigma_p\sigma_b$ to the right-hand side of equation 1, and the complete form of equation 2 is obtained by adding the term $2r\overline{p}\overline{b}\sigma_{\overline{p}}\sigma_{\overline{b}}$ to the right-hand side of equation 2, in which r is the coefficient of correlation between the percentage of infestation and mean number of borers per infested plant in the quarters of the field and $\sigma_{\overline{p}}$ and $\sigma_{\overline{b}}$ are the standard errors of the means of the two factors. It should be noted that in the complete form of the equations as applied to this problem there would be only four observations for both p and b. Therefore $n'_p=n'_b=4$. The use of the complete form of equation 2 gives an estimate of the variance of the mean of the products from the quarters of the field.

SAMPLING VARIABILITY WITHIN 20 FIELDS IN WOOD COUNTY, OHIO

Table 2 gives the results of the calculations for each field of the 1932 survey of Wood County, Ohio, and serves to illustrate the process carried out for each field in the other counties of the 1931 and 1932 surveys. As an intermediate step in making the substitutions in equation 2, column 9, a, of table 2 gives the value of the term of equation 2 for $n'_b=1$, column 9, b, gives the value of the term for $n'_p=1$, while column 9, c, gives the variance of their product. The values in columns 9, a, and 9, b, may now be divided by the values of n'_b and n'_p, respectively, representing the actual number of samples taken in the respective fields, to obtain the values given in columns 10, a, and 10, b. Column 10, c, the sum of columns 10, a, and 10, b, gives the variance of a product when σ^2/n'_b and σ^2/n'_p are the variances of the means $\overline{b}$ and $\overline{p}$.

TABLE 2.—*Data on European corn borer infestation in 20 fields in Wood County, Ohio, in 1932, illustrating the details of the calculations for obtaining the sampling variance of the mean number of borers per 100 plants in each field*

Field no.	Statistical constants for number of infested stalks out of 100 stalks			Statistical constants for number of borers per infested stalk				The variance of a product when σ_b^2 and σ_p^2 are the variances of a single observation ($\overline{p}^2\sigma_b^2+\overline{b}^2\sigma_p^2=\sigma_{\bar{x}}^2$)			The variance of a product when σ^2/n'_b and σ^2/n'_p are the variances of the means $\overline{b}$ and $\overline{p}$ ($\overline{p}^2\sigma^2/n'_b+\overline{b}^2\sigma^2/n'_p=\sigma_{\bar{x}}^2$)			Standard error of product ($\sigma_{\bar{x}}$)	Field mean number of borers per 100 plants
	Mean infested stalks ($\overline{p}$)	Standard deviation (σ_p)	Standard error of mean ($\sqrt{\frac{\sigma_p^2}{4}}$)	Mean borers per stalk ($\overline{b}$)	Size of sample (n'_b)	Standard deviation (σ_b)	Standard error of mean ($\sqrt{\frac{\sigma_b^2}{n'_b}}$)	(a)	(b)	(c)	(a)	(b)	(c)		
(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)			(10)			(11)	(12)
1	15	3.8	1.9	1.3	10	0.82	0.26	151	+ 24	= 175	15	+ 6	= 21	4.6	19.5
2	15	7.3	3.6	1.7	10	1.42	.45	454	+ 154	= 608	45	+ 39	= 84	9.2	25.5
3	33	15.1	7.5	1.7	10	1.34	.42	1,955	+ 659	= 2,614	196	+ 165	= 361	19.0	56.1
4	8	10.8	5.4	1.1	8	.84	.30	46	+ 141	= 187	6	+ 35	= 41	6.4	8.8
5	33	24.7	12.3	2.1	10	1.73	.55	3,259	+ 2,690	= 5,949	326	+ 673	= 999	31.6	69.3
6	38	15.1	7.5	2.4	10	1.43	.45	2,953	+ 1,313	= 4,266	295	+ 328	= 623	25.0	91.2
7	46	23.7	11.8	1.3	10	1.34	.42	3,799	+ 949	= 4,748	380	+ 237	= 617	24.8	59.8
8	60	11.3	5.6	1.4	10	.84	.27	2,540	+ 250	= 2,790	254	+ 63	= 317	17.8	84.0
9	6	9.5	4.7	1.5	6	1.38	.56	69	+ 203	= 272	12	+ 51	= 63	7.9	9.0
10	12	7.8	3.9	1.2	10	.79	.25	90	+ 88	= 178	9	+ 22	= 31	5.6	14.4
11	4	3.3	1.6	1.5	4	1.00	.50	16	+ 25	= 41	4	+ 6	= 10	3.2	6.0
12	6	2.3	1.1	1.8	6	.98	.40	35	+ 17	= 52	6	+ 4	= 10	3.2	10.8
13	21	8.9	4.4	2.5	10	2.17	.69	2,077	+ 495	= 2,572	208	+ 124	= 332	18.2	52.5
14	47	11.0	5.5	2.8	10	1.62	.51	5,797	+ 949	= 6,746	580	+ 237	= 817	28.6	131.6
15	27	15.1	7.5	2.1	10	1.20	.38	1,050	+ 1,006	= 2,056	105	+ 252	= 357	18.9	56.7
16	49	17.4	8.7	2.8	10	2.15	.68	11,099	+ 2,377	= 13,476	1,110	+ 594	= 1,704	41.3	137.2
17	80	9.8	4.9	2.1	10	1.45	.46	13,456	+ 424	= 13,880	1,346	+ 106	= 1,452	38.1	168.0
18	37	8.9	4.4	1.6	10	1.08	.34	1,597	+ 203	= 1,800	160	+ 51	= 211	14.5	59.2
19	79	6.8	3.4	1.5	10	1.35	.43	11,374	+ 104	= 11,478	1,137	+ 26	= 1,163	34.1	118.5
20	56	31.5	15.7	2.7	10	1.57	.50	7,730	+ 7,234	= 14,964	773	+ 1,809	= 2,582	50.8	151.2
Sum								69,547	+ 19,305	= 88,852	6,967	+ 4,828	= 11,795		1,329.3
Mean								3,477.35	+ 965.25	= 4,442.60			589.75		66.47

SAMPLING VARIABILITY WITHIN ALL FIELDS OF THE SURVEY, GROUPED ACCORDING TO LEVEL OF BORER POPULATION

In order to generalize as completely as possible the information from the entire survey, the fields of all counties were grouped according to the ranges shown in table 3 and by adding and averaging the field means and the variances for $n'_b=1$ and $n'_p=1$ of each term and the totals for the variance of a product. The ranges used in this generalization are entirely arbitrary, table 3 giving a final summary of the variances by field level as finally used. (Fields with means of 4.9 borers per 100 plants or lower are omitted.) In this table is condensed all the information the data afford as to the average magnitude and relationships of the parts of the variance at the different levels of field populations. Each field, representing its own peculiar set of conditions, has its proportionate effect on the final results. The result is not universal but is applicable insofar as these data constitute a representative sample of a given set of conditions encountered in these surveys.

TABLE 3.—*Summary of the sampling variance within fields, grouped within certain ranges of the estimates of the mean number of European corn borers per 100 plants, in the counties of the surveys of 1931 and 1932 in the 1-generation area*

Group no.	Borers per 100 plants (range)	Fields	Borers per 100 plants (group mean)	Variance of a single observation ($\bar{p}^2\sigma_b^2+\bar{b}^2\sigma_p^2=\sigma_x^2$) (5)			Standard deviation (σ_x)	Coefficient of variation
(1)	(2)	(3)	(4)	(a)	(b)	(c)	(6)	(7)
		Number	*Number*				*Number*	*Percent*
1	5.0- 8.9	154	6.7	26.8	+ 61.7	= 88.5	9.4	140.3
2	9.0- 14.9	165	11.7	72.1	+ 121.2	= 193.3	13.9	118.8
3	15.0- 24.9	145	19.5	195	+ 210	= 405	20.1	103.1
4	25.0- 34.9	112	29.6	425	+ 321	= 746	27.3	92.2
5	35.0- 44.9	78	40.2	759	+ 499	= 1,258	35.5	88.3
6	45.0- 54.9	52	49.8	1,341	+ 670	= 2,011	44.8	90.0
7	55.0- 74.9	100	65.0	2,180	+ 989	= 3,169	56.3	86.6
8	75.0- 94.9	57	83.9	3,528	+1,129	= 4,657	68.2	81.3
9	95.0-124.9	80	109.0	5,289	+1,637	= 6,926	83.2	76.3
10	125.0-154.9	31	137.7	8,535	+2,521	= 11,056	105.1	76.3
11	155.0-194.9	32	171.0	13,974	+2,373	= 16,347	127.9	74.8
12	195.0-234.9	19	211.4	21,775	+3,947	= 25,722	160.4	75.9
13	235.0-274.9	7	254.7	31,047	+3,329	= 34,376	185.4	72.8
14	275.0-324.9	8	295.7	48,968	+2,747	= 51,715	227.4	76.9
15	325.0-374.9	3	354.2	47,357	+2,577	= 49,934	223.5	63.1
16	375.0-499.9	6	423.0	73,920	+2,560	= 76,480	276.6	65.4
17	500.0-699.9	3	525.9	80,167	+3,115	= 83,282	288.6	54.9
18	700.0 up	2	970.0	180,222	+ 0	=180,222	424.5	43.8

Table 3 may be inspected to determine the general characteristics of the sampling variability within the fields. In column 6 are given the generalized standard deviations of the products corresponding to their variances in column 5, c. Column 7 contains the standard deviations expressed as percentages of the generalized means of column 4. These relative values of the standard deviations are statistical constants known as coefficients of variation; they serve as indexes of variability and measure the fluctuation within the fields at the different levels of borer population.

It will be noted that the standard deviations increase in actual magnitude as the means increase, but decrease steadily in relative magnitude from about 40 percent larger than the mean at the lowest

level to less than 50 percent of the mean at the highest level. The part of the variance of the product represented by the term $\bar{b}^2\sigma_p^2$ in column 5, b, of table 3 increases until the mean population reaches 211.4 borers per 100 plants. It then decreases, becoming zero at the highest level. The variance of the infestation samples, the σ_p^2 part of this term, decreases as the limit of 100-percent infestation is approached. As the square of the mean number of borers per infested plant, the $\bar{b}^2$ part of the term, increases it tends to increase the value of the term, but not at a rate sufficiently great to offset the reduction of the value of the variance of the infestation samples as the limit of 100 percent infestation is approached. At 100-percent infestation the variance of the infestation samples becomes zero and the term vanishes completely.

The part of the variance of the product represented by the term $\bar{p}^2\sigma_b^2$ in column 5, a, of table 3 is the dominant influence in determining the total variance above a level of about 25 borers per 100 plants. This term increases from 57.0 percent of the total variance at a level of about 30 borers to about 96 percent of the total at a level between 400 and 500 borers per 100 plants. Over the range of the populations covered in these data the variability in the number of borers in the infested plants, the σ_b^2 part of this term, continues to increase as the mean number of borers per infested plant, $\bar{b}$, increases. The square of the number of infested plants per 100, the $\bar{p}^2$ factor of this term, increases up to 10,000 as $\bar{p}$ increases to its maximum of 100. However, increases in population beyond 100 percent infestation must be as a result of increased number of borers per infested plant, which means that any increase in this term is wholly dependent on the magnitude of the σ_b^2 factor, while the $\bar{p}^2$ factor remains constant.

Relative Values of Different Combinations of n'_b and n'_p

Because of this change in the relative as well as the actual variation at different levels of infestation, it is necessary to study the relative values of different combinations of n'_b and n'_p in sampling at each population level. Three population levels, 11.7, 109.0, and 423 borers per 100 plants were selected from table 3 for study. By substituting in equation 2 the corresponding values from columns 5, a, and 5, b, table 3, and also different combinations of values of n'_b and n'_p, the variances of a product for the various sampling combinations for each of the selected levels may be computed. The variances of a product were calculated for all combinations of n'_b and n'_p equal to 1, 2, 3, 4, 5, 7, and 10 for the three selected levels. The standard deviations were then expressed as percentages of the respective means, i. e., as coefficients of variation. The results are shown in the graphs of figure 1.

As noted previously, although the actual magnitude of the standard deviation of the mean for any sampling combination increased as the mean level of population increased, the relative magnitude decreased. Thus, the surfaces of the three graphs of the standard deviations in terms of percent of the mean are increasingly lower as the means increase.

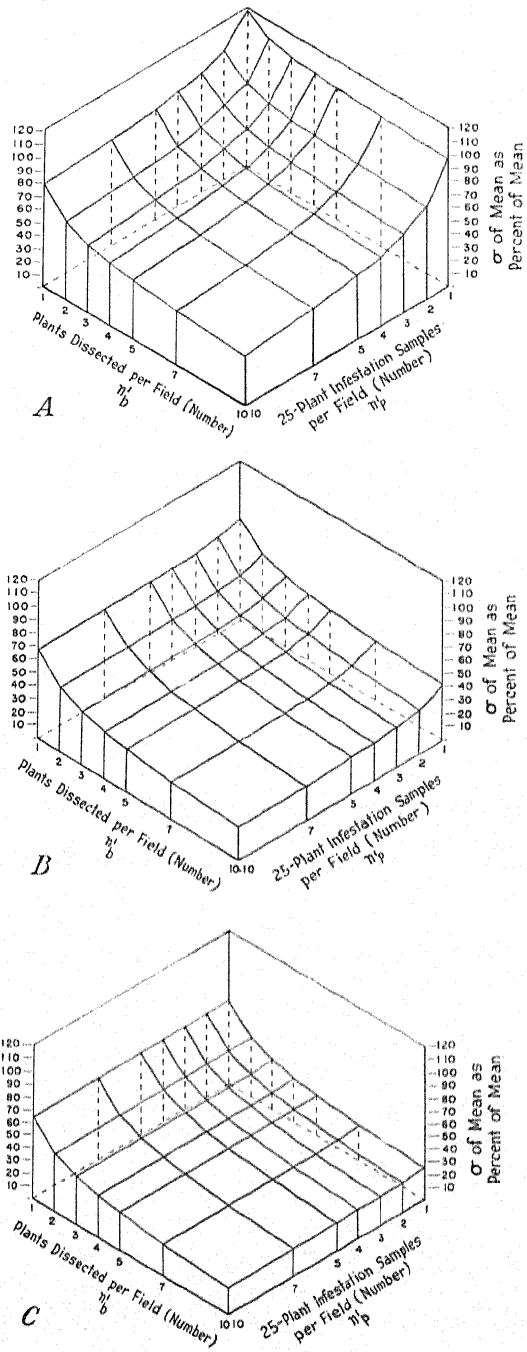


FIGURE 1.—Standard deviations of the field means as percentages of means for different combinations of sampling within fields when field means approximate: *A*, 11.7 borers per 100 plants; *B*, 109.0 borers per 100 plants; *C*, 423.0 borers per 100 plants.

At the level of 11.7 borers per 100 plants (fig. 1, *A*), the second term of the equation (column 5, b, table 3), which is reduced as the number of 25 plant-infestation samples, n'_p, is increased, is larger than the first term (column 5, a, table 3), which is reduced as the number of plants dissected, n'_b, is increased, so that the total variance is affected more by a comparable increase in the number of infestation samples taken than in the number of plants dissected. At this level the taking of two infestation samples instead of one sample reduces the total variance almost as much as if seven plants had been dissected instead of one plant. However, the predominant effect of taking additional infestation samples on reducing the total variance is soon lost, for at the level of 19.5 borers the taking of two infestation samples instead of one sample reduces the total variance only slightly more than if two plants had been dissected instead of one plant. At the level of 109.0 borers per 100 plants (fig. 1, *B*) the taking of two infestation samples instead of one sample reduces the total variance only about a third as much as if two plants had been dissected instead of one plant, while at the level of 423.0 borers per 100 plants (fig. 1, *C*) the effect is about one-thirtieth as much. At the level of 423.0 borers per 100 plants, increasing the number of infestation samples above two has practically no effect whatever in reducing the sampling variability of the estimate of the mean in such fields.

OTHER CONSIDERATIONS OF SAMPLING WITHIN FIELDS

In any survey numerous restrictions are always placed on the plan of sampling, solely for eliminating bias rather than because of purely quantitative aspects of the sampling variability. In sampling within the fields in the present survey one such restriction concerned the infestation sample of 25 consecutive plants. Smaller total numbers of plants will suffice for attaining a given degree of reliability in the estimation of the mean percentage of infestation if smaller numbers of consecutive plants are taken at each place selected at random and examined within a field. For example, compared with taking a total of 100 plants, each plant at a place selected at random, it was found that to attain a given reliability in the estimation of the mean percentage of infestation, 171 plants were required when 5 consecutive plants were taken in a place, 206 when 10 plants were taken, 285 when 25 plants were taken, and 660 plants when 100 plants were taken. These results were obtained as an average for five fields with percentages of infestation averaging 38.6, no field having been infested more than 50 percent. Taking the foregoing numbers of plants in the designated manner gave a standard error of 4.71 to the mean of 38.6 percent, but the same proportions would hold among the total numbers of plants required to attain any other degree of reliability in the estimation of the mean of these five fields.

To take each plant of the total number examined for infestation in the fields of this survey in a place selected at random, however, would have necessitated a scheme to eliminate all possible conscious or unconscious bias in taking the plants. Any such scheme would probably be difficult to devise as well as impractical on account of the time necessarily consumed in putting it into effect. For this reason samples of 25 consecutive plants taken in each quarter of the field were arbitrarily chosen from which to determine the percentage of infestation, in the belief that samples of this size would render bias negligible.

The question of the randomness of these samples is essentially the same as that involved in the technique of determining the yield of plots or fields of small grains by harvesting small areas within them. Clapham (*1, 2, 3*) and Kalamkar (*6*) studied such problems and presented experimental data, and on the basis of the analysis of the variance deemed the placement of the samples at definite intervals in the same drill rows to be undesirable. Rather, they recommended that the area be divided into a definite number of equal-sized areas and the samples taken at random in these. The objections noted by these authors to definite placement of samples hardly apply to the manner of taking the infestation samples at approximately the center of the quarters of the fields. These samples were taken without regard to the condition of the corn at the place of sampling in the fields and as an average of a large number of fields the variability would approximate closely to that of fully randomized samples within the quarters of the fields which correspond to sections as recommended by the foregoing authors.

Methods of avoiding bias when taking the infested plants for dissection to determine the number of borers per infested stalk also had to be considered. For this reason only infested plants were dissected in the infestation samples, up to the maximum number specified for the fields as stated previously. It is obvious that in the field the most heavily infested plants would be the ones most easily found, and unless careful safeguards were taken in the methods of the survey to obtain representative plants from the whole field a considerable bias would be introduced.

The most important question in sampling within the fields, however, is the relative weight that should be given to the samples of the two sets of factors and this question is answered by the solutions carried out on the basis of the equation for the variance of products. As used also, the actual sampling variability within the fields, while important in properly planning area-wide surveys, is distinctly subordinate to the field-to-field variability within the larger areas, in its final effect on the reliability of the estimate of the county mean number of borers per 100 plants.

ANALYSIS OF THE FIELD-TO-FIELD VARIABILITY WITHIN COUNTIES

METHODS OF ANALYSIS OF VARIANCE APPLIED TO SAMPLING VARIABILITY OF AREA-WIDE SURVEYS

The foregoing analysis treated of the sampling variability within the fields so that estimates could be obtained of the true levels of infestation of each field, these in turn being used to obtain estimates of the mean levels of infestation of the counties. Within the counties the actual level of infestation will vary greatly from field to field. The differences between the fields constitute another component of the variability affecting the reliability of the estimates of the mean populations of the county areas, and this component is of paramount importance in the specification of the methods of sampling to be used in the surveys. To measure and study the characteristics of this component of the variability by itself, and in comparison with the components of the sampling variability within the fields, actual observations under the conditions to be studied must be used. The data for the field estimates of the mean number of borers per 100 plants

for the 66 counties already used for the study of the sampling variability within the fields constitute the data used for this study of the field-to-field variability within counties.

The fields sampled were selected at predetermined points distributed fairly uniformly over the county, thus insuring, to the greatest possible degree, both representativeness and randomness in the samples. The first step is to determine by the methods of the analysis of variance devised by Fisher (*4*) whether the fields within sections of the counties tended to be more nearly alike in level of infestation than the fields over the whole counties—in other words, to see whether there were significant differences in level of infestation between the sections of the counties. For this study each county was divided into four sections of approximately equal area but more or less arbitrarily delimited so as to have each section include the same number of fields in as compact units as possible. The allocation had to be done after the surveys were carried out, but care was exercised to avoid any consideration of the values in making up the sections, these being based entirely on location. In counties in which 20 fields were sampled each section included 5 fields and in counties in which 25 fields were sampled each section included 6 fields. (Fields numbered 13 in the counties of 25 fields were uniformly and arbitrarily discarded and county means and other values adjusted accordingly.) The solutions were carried out by the methods of the analysis of variance to determine (1) the total variation of the field means from the county mean, (2) the variation of the field means within the sections from the means of their respective sections, and (3) the variability attributable to the differences between the mean population levels in the four sections. These parts were later related to the sampling variability within fields, solved in the preceding section of the paper.

DATA FOR SURVEY OF WOOD COUNTY, OHIO, IN 1932 USED TO ILLUSTRATE THE SOLUTION

The values for the mean numbers of borers per 100 plants for the 20 fields surveyed in Wood County in 1932 will be used to illustrate the solutions which were applied to all counties. These values are given in column 12 of table 2 and the manner of grouping the fields into the four sections is shown in figure 2; while the values, arranged according to sections for the analysis of the variance, are given in table 4.

TABLE 4.—*Mean number of European corn borers per 100 plants by fields for the survey of Wood County, Ohio, in 1932, arranged in sections for the analysis of the variance*

Section 1		Section 2		Section 3		Section 4	
Field no.	Mean number borers	Field no.	Mean number borers	Field no.	Mean number borers	Field no.	Mean number borers
1	19.5	3	56.1	10	14.4	12	10.8
2	25.5	4	8.8	15	56.7	13	52.5
5	69.3	8	84.0	16	137.2	14	131.6
6	91.2	9	9.0	17	168.0	19	118.5
7	59.8	11	6.0	18	59.2	20	151.2
Total	265.3		163.9		435.5		464.6

Examples of the application of the methods of the analysis of variance to similar problems and detailed discussions of the concepts involved are given in numerous publications including Fisher (*4*), Wishart (*9*), and Snedecor (*8*). Although all these vary somewhat in the particular arithmetic procedure, the basic concepts are the same.

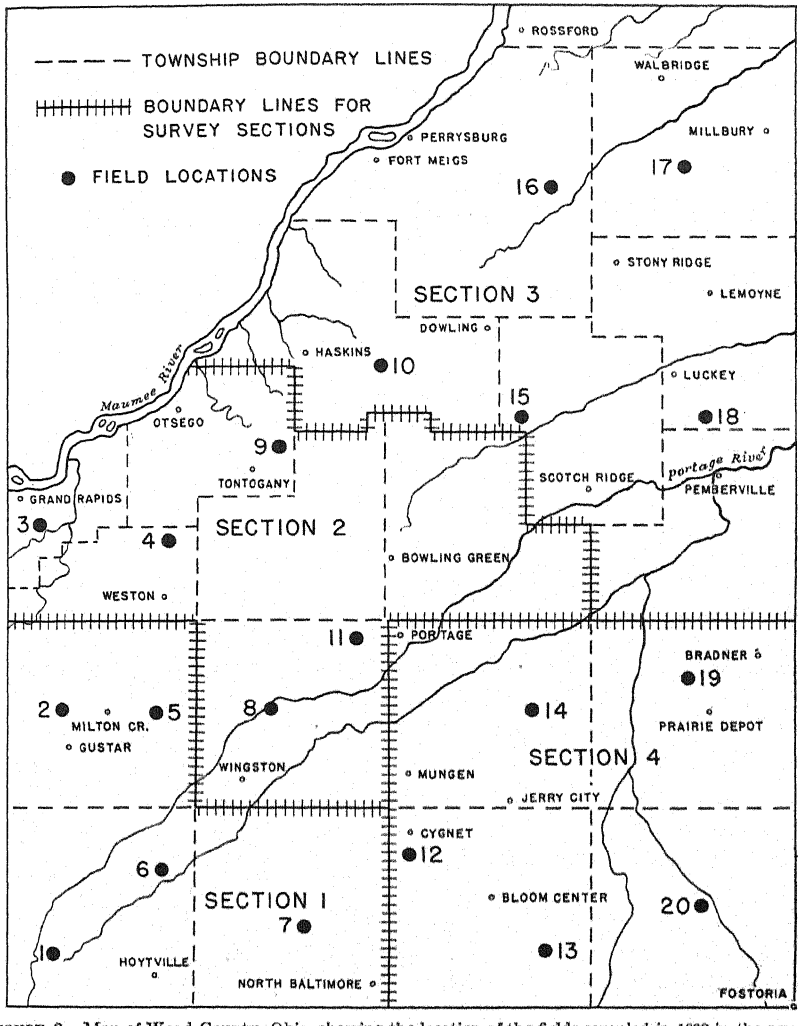


FIGURE 2.—Map of Wood County, Ohio, showing the location of the fields sampled in 1932 in the survey of the number of borers per 100 plants, and the allotment into four sections for the analysis of the field-to-field variability within the county.

Specifically, this particular problem is identical with the class given by Snedecor (*8*) as example 1, page 3, of his manual. This type of problem has a single criterion of classification and an equal number of observations in each of the classes.

From table 5 it will be noted that the sum of the squares of the deviations of the 20 field means from the section means repre-

senting 16 degrees of freedom is less than the sum of the squares of the deviations of 20 field means from the county mean representing 19 degrees of freedom. The difference is attributable to the 3 degrees of freedom representing the deviations of the population levels of the four sections from the general county mean.

TABLE 5.—*Analysis of the variance in the mean number of European corn borers per 100 plants in Wood County, Ohio, in 1932*

Variation	Degrees of freedom	Sum of squares	Mean-square deviation	Standard or root-mean-square deviation
Total for county	19	50,842.07	2,675.90	51.73
Within sections	16	38,641.85	2,415.12	49.14
Between sections	3	12,200.22	4,066.74	63.77

The values in the last two columns of this solution for the mean-square deviation (variance) and the root-mean-square deviation are directly comparable in magnitude within the columns.

Assuming that real differences were indicated between the actual population levels of the sections as laid out in Wood County in 1932, the mean-square deviation or variance between sections in table 5 may be considered as being made up of two parts, the first with variance of A_1, representing the estimated actual differences in level of population between sections, and the second or B_1 part, which is the mean of the variance within sections for the individual fields shown above. This phase of the problem has been discussed by Fisher (*4*) in section 40. The A_1 part is found from the difference between 4,066.74 and 2,415.12, or 1,651.62, while the B_1 part is the mean variance between fields within sections, or 2,415.12. The values of the mean-square deviation in table 5 are the variances of single fields, and since a section comprises 5 fields, the A_1 part of the variance of the section mean number of borers per 100 plants is one-fifth of 1,651.62, or 330.32. The square root of this, 18.17 borers per 100 plants, is the estimated standard error of the real levels of population from section to section, corrected for the sampling variability within the sections. This discussion is more readily understood by assuming that the mean-square deviation between sections approximates the mean-square deviation within sections so closely that the difference between them is well within the limits set for errors of random sampling. Then the variability between fields in one section and fields in other sections would not be significantly greater on the average than the variability from field to field within the sections and the A_1 part of the variance would not exist.

But the A_1 part of the variability within counties is irrelevant to the reliability of the estimates of the county means if at least one field is sampled in each section, and it should be eliminated in the estimate of the true sampling variability. In replicated field experiments Fisher (*5*) terms this method "local control", but it can be applied as well to methods of area-wide insect surveys. It is evident, therefore, that only the B_1 part, or the variance of the field means within the sections, applies to the reliability of the estimates of the county

means. In the solution as carried out here the local control is applied by dividing the county into four sections. The 20 or 25 fields sampled in these surveys were distributed over the counties, but were arbitrarily grouped into 4 sections as being the solution combining most advantages in the study. In order to carry out any formal solution for the estimate of the parts of the variance, there must be at least two assumed sections and at least two fields in each section. If there are the same number of sections in each county the results may be combined for study later. Also, in the study hereinafter carried out, if there are four sections per county the estimated variability of county means will proceed by four-field intervals, which is a convenient unit.

Just as the total variability of the field means from the county mean was separated into two parts, so also may the B_1 part of the variance which applies to the reliability of sampling in the county be divided into two parts for more detailed study of the sampling methods. The first part, designated as the A_2 part, represents the estimated variance of the real levels of population of the fields within the sections, and the second part, designated as the B_2 part, represents the variability of the sampling within the fields for the estimation of the field means and will be taken at $n'_p = n'_b = 1$. This B_2 part is obtained directly from column 9, c, in table 2. The total 88,852 is divided by the number of fields (20) sampled in the county, which gives a mean-square deviation of 4,442.6. The square root of this value, 66.65 borers per 100 plants, is the corresponding standard deviation. The components of this 4,442.6, affected respectively by modifications in the numbers of plants dissected to determine the number of borers per infested plant and in the numbers of samples taken to determine the percent infestation, are obtained in the same manner from the corresponding columns, i. e., 9, a, and 9, b, of the same table. The mean-square deviation affected by the number of plants dissected is 3,477.35 and that affected by the number of infestation samples is 965.25, designated as the C_2 and D_2 parts of the variance, respectively.

The A_2 part is equated from the B_1 part as the A_1 part was equated from the variance of the section means. The B_1 part, or the variability of the field means within the sections, is made up of a part, designated as the A_2 part, which represents the difference of the true field levels from the section levels, and a part due to the variability of the sampling within the fields. The latter is estimated from column 10, c, of table 2. This column gives the estimated total variance of sampling within each field for the actual sampling combination used. Each field has to be figured separately owing to the differences in the number of plants dissected in the various fields, otherwise this step could be figured in one operation, as in subtracting the B_1 part from the variance between sections for estimating the A_1 part of the variance. The sum of this column, 11,795, divided by 20 gives the average variance within the fields, or 589.75. This is subtracted from the variance within sections, B_1, or 2,415.12, giving as A_2 1,825.37, or the estimated variance of the true levels of population of the fields from the section levels; that is, it gives the estimated variance if all field levels and section levels were known without error. The square root of this value, 42.72 borers per 100 plants, is the corresponding standard deviation among the fields within sections.

The sampling within the fields, however, contributes to the variability of the fields within the counties and thus influences the reliability of the county means obtained as estimates of the true population levels. The B_2 given above for $n'_p=n'_b=1$, and its parts, C_2 and D_2, are taken for the basic estimate of these values for counties for the purpose of standardization. The sum of B_2 and A_2 is the estimated total variance of fields within sections when 1 sample of 25 consecutive plants is examined to determine the percentage of infestation, and 1 infested plant is dissected to determine the number of borers per infested plant in each field. The square root of this value, 79.17 borers per 100 plants, is the corresponding total standard deviation for fields within sections.

This completes the analysis of the variability of the samples as actually taken in the county. The information that has been derived is summarized in table 6.

TABLE 6.—*Summary of analysis of the variance in surveys of European corn borer populations, Wood County, Ohio, 1932*

Estimated variability of—	Variance		Standard deviation, borers per 100 plants
	Designation	Value	
			Number
Section levels from county level	A_1	330.32	18.17
Fields levels from section level	A_2	1,825.37	42.72
Sampling within fields ($n'_p=n'_b=1$)	B_2	4,442.60	66.65
Total of estimates of field means ($n'_p=n'_b=1$) from section means	A_2+B_2	6,267.97	79.17

SAMPLING VARIABILITY FOR THE COUNTIES GROUPED BY LEVEL OF POPULATION

An analysis similar to that made in Wood County, Ohio, was made for each of the other 65 counties of the 1931 and 1932 surveys in the one-generation area in which the sampling variability within fields was studied. The counties were then arranged according to the mean numbers of borers per 100 plants, and the variances added and averaged over specified ranges as shown in table 7. The ranges used here are entirely arbitrary, as in the case of the ranges used for the study of the variability of the sampling within fields. Corresponding standard deviations, both as actual values and as percentages of the group means of the numbers of borers per 100 plants, are also given in the table.

TABLE 7.—*Summary of the sampling variance within counties of the estimates of the mean number of European corn borers per 100 plants for the counties of the 1931 and 1932 fall surveys of infestation of the one-generation area studied*

Group no.	Borers per 100 plants (range)	Counties	Borers per 100 plants (group mean)	Mean variances for—				Standard deviations for—								Variances affected by—	
				Section levels from county levels (A_1)	Field levels from section levels (A_2)	Sampling within fields (B_2)	Total (A_2+B_2)	Section levels from county levels		Field levels from section levels		Sampling within fields		Total		Number of plants dissected (C_2)	Number of infestation samples (D_2)
								$\sqrt{A_1}$	As percent of mean	$\sqrt{A_2}$	As percent of mean	$\sqrt{B_2}$	As percent of mean	$\sqrt{A_2+B_2}$	As percent of mean		
	Number	*Number*	*Number*					*Number*	*Percent*	*Number*	*Percent*	*Number*	*Percent*	*Number*	*Percent*		
1	1– 4.99	6	2.68	0.49	3.7	30.5	34.2	0.70	26	1.92	72	5.52	206	5.85	218	8.3	22.2
2	5– 9.99	6	8.88	11.1	56.0	184.2	240.2	3.33	38	7.48	84	13.57	153	15.50	175	86.2	98.0
3	10– 19.99	12	13.60	23.6	139.0	366.5	505.5	4.86	36	11.79	87	19.14	141	22.48	165	193.2	173.3
4	20– 29.99	8	24.71	62.9	603.1	943.5	1,546.6	7.93	32	24.56	99	30.72	124	39.33	159	649.1	294.4
5	30– 44.99	6	36.59	39.0	905.6	1,871.5	2,777.1	6.24	17	30.09	82	43.26	118	52.70	144	1,367.8	503.7
6	45– 64.99	7	57.55	730.0	2,734.2	3,965.7	6,699.9	27.02	47	52.29	91	62.97	109	81.85	142	3,032.4	933.3
7	65– 79.99	13	70.75	578.7	4,439.9	5,632.7	10,072.6	24.06	34	66.63	94	75.05	106	100.36	142	4,742.2	890.5
8	80–119.99	5	99.61	1,688.2	9,153.4	10,197.4	19,350.8	41.09	41	95.67	96	100.98	101	139.11	140	8,934.2	1,263.2
9	120 and up	3	158.74	[1]	32,394.6	20,371.6	52,766.2	[1]	--------	179.98	113	142.73	90	229.71	145	18,482.3	1,889.3

[1] None.

Examination of the results in table 7 reveals that the total standard deviation relevant to the sampling of the county means, $\sqrt{A_2+B_2}$, increases in actual magnitude as the mean level of population increases in the counties. This standard deviation also decreases in relative magnitude but with no pronounced trend at the higher levels of borer population. The standard deviation of the A_1 part of the variance, which is the estimated variance of the real section levels from the county levels of population and is eliminated by the principle of local control, and therefore is not relevant to the reliability of the estimates of the county means, increases in actual magnitude as the mean level of population increases in the counties. The percentage this standard deviation bears to the county means is almost constant. The standard deviation of the A_2 part of the variance, which is the estimated variance of the real field-to-field differences within sections, increases in both actual and relative magnitude as the county levels of population increase.

The standard deviation of the B_2 (for $n'_p=n'_b=1$) part of the variance, which is the variance of sampling within the fields, increases in actual magnitude and decreases in relative magnitude as the county levels increase. Within the fields the parts of the B_2 variance C_2 (which is affected by the number of plants dissected, n'_b) and D_2 (which is affected by the number of infestation samples, n'_p) show somewhat the same characteristics on the basis of counties as they did for the individual fields, when classified by population levels. Both parts increase in actual magnitude as the county levels increase, but the C_2 part increases very much more rapidly than the D_2 part.

These characteristics of the parts of the variability of sampling within the counties all influence the relative effectiveness of different methods of sampling in estimating the levels of populations in various areas. Figure 3 shows the standard deviations of county means for various sampling combinations for the three levels 8.88, 57.55, and 99.61 borers per 100 plants taken from table 7.

The values for figure 3 were derived as follows: Let n' equal the number of sections per county (in this analysis assumed to be 4) and let k equal the number of fields sampled per section. Then the variance of the estimates of the county means for any sampling combination is given by the equation

$$\sigma^2 \text{ county mean} = \frac{A_2+\frac{C_2}{n'_b}+\frac{D_2}{n'_p}}{n'k}. \qquad \text{Equation 5}$$

As the number of fields sampled in a county is increased over four the area is divided into an equal number of sections of uniform size and one sample taken in each to accomplish the greatest possible amount of local control, thus shifting more of the A_2 part of the variance to the A_1 part, but it was not practical with these data to carry out a solution which conformed strictly to this principle. Finally, the standard errors of the means for the various sampling combinations are stated as percentages of the corresponding means. Corresponding values for all sampling combinations for $n'=4$ with k values from 1 to 10 (4 to 40 fields) and $n'_b=1$ and 5, and $n'_p=1$ and 4 were computed for the three levels shown in figure 3.

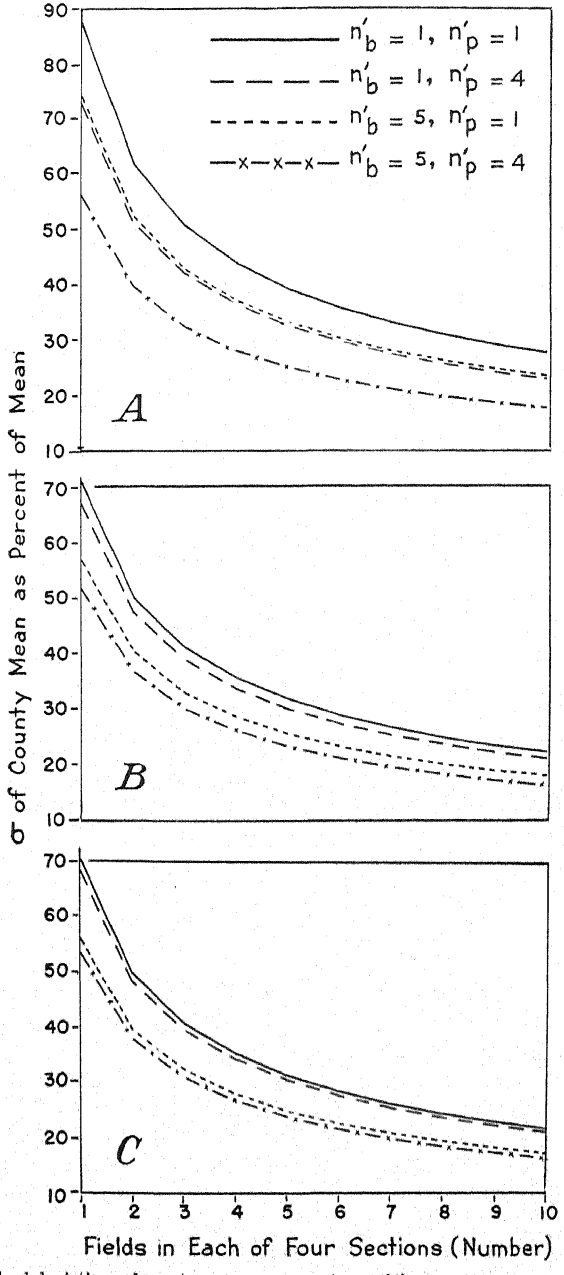


FIGURE 3.—Standard deviations of county means as percentages of the county means for various combinations of samples within the fields and various numbers of fields sampled within the county for means of: *A*, 8.88 European corn borers per 100 plants; *B*, 57.55 borers per 100 plants; *C*, 99.61 borers per 100 plants.

At the level of 8.88 borers per 100 plants, increasing the number of plants dissected, n'_b, from 1 to 5 within each field has nearly the same effect in reducing the standard deviation of the estimates of the county means as increasing the number of 25-plant infestation samples, n'_p, from 1 to 4, and increasing both n'_b and n'_p by these amounts at the same time is about twice as effective. As the levels of population of the counties increase, the reduction in the variability of the county means due to increasing the number of infestation samples decreases rapidly, and the curve for $n'_b=1$ and $n'_p=4$ approaches the curve for $n'_b=1$ and $n'_p=1$, and the curve for $n'_b=5$ and $n'_p=1$ approaches the curve for $n'_b=5$ and $n'_p=4$. The characteristics of the various parts of the variability of sampling in this survey are thus revealed.

DISCUSSION

In the past, several studies have been conducted relative to the methods of sampling to be followed within fields in surveys of insect abundance. From the standpoint of the methods of conducting area-wide surveys those studies give information only on a relatively less important part of the total variance of sampling, whereas they really should apply to the estimates of the mean of the larger areas. Furthermore, the fields in which the sampling studies were conducted often were selected and limited in number, thus still further limiting the applicability of the results. It is only from actual survey data, such as are used in this paper, that estimates of all the essential parts of the sampling variability can be obtained. This information on the characteristics of the sampling variability in the surveys is obtained along with the current collection of information on the status of the insect population. The specifications which effectively meet these two requirements in a survey are practically the same.

The method of sampling at the beginning of a survey must be based on such information as may be at hand, including the knowledge of the biology of the insect and host, matters of agronomy, the general principles of survey sampling, the objects of the survey, similarity to other surveys that have been conducted, and the reconnaissance observations that have been carried out previously on the same problem. It should not be difficult to arrive at specifications fairly near the most efficient ones possible by making use of all these factors. As the data accumulate in the survey they are analyzed in reasonable detail and the desirable improvements in methods inaugurated.

The three basic principles of survey sampling which are essential to meet the requirements of obtaining the most reliable estimate of the area means, and the proper measurement of the parts of the sampling variable, are: (1) Replication or multiple samples in each subdivision of the survey (i. e., counties or fields); (2) local control, or dividing the area to be sampled (counties or fields) into as many sections, uniform in size or representing aliquot parts of the host organism to be sampled, as there are samples to be taken; and (3) randomness, or taking one of the specified samples at random in each section.

Replication increases the reliability of the estimates of the area means, such as counties or fields, directly in proportion to the number of samples. The larger the number of samples taken, therefore, the greater the reliability of the estimate of the mean, although as the number becomes larger the relative effectiveness of this factor per unit sample becomes less and less. When combined with the principle of local control, however, the effectiveness of the replication is usually greatly enhanced.

The sampling variability which applies to the reliability of the estimates of the means is the variability within sections for each phase of sampling (county or fields in this survey). As the number of sections is increased, more and more of the total variability of the given phase is shifted from the part of the variability from sample to sample within the sections, that which is relevant to the variability of the estimates of the means (*5*), to the part of the variance for differences between the levels of the sections, that which is irrelevant to the variability of the estimates of the means. Thus, by the combination of the principles of multiple samples and local control, the variability of the estimates of the corresponding means is reduced much more rapidly than the square root of the number of samples operating on the total variability of the unit samples. If for any reason the samples are grouped in clusters, some of the advantage of local control is lost and the solution for the analysis of the variance must be modified accordingly. This is true even though it is necessary to consider two or more of the samples of a given area of the survey together in a section of correspondingly larger size for the analysis of the variance. Some of the true section-to-section variability which is irrelevant to the variability of the estimates of the means is of necessity included within sections because of this limitation in the solution for actual survey data, but the entire gain due to local control by placing all samples in uniform sections actually is inherent in the estimate of the mean itself. The discrepancy usually is not very great. Randomness in taking the samples within the section is, of course, absolutely essential for obtaining either an unbiased estimate of the corresponding means or a correct estimate of the sampling variability.

Although these principles of sampling apply equally to sampling within fields or between the fields of a county, the phases differ greatly in relative effectiveness, per unit sample, on the reliability of the estimates of the means. Increasing the number of samples of the phases involving successively larger unit areas has much more effect on the reliability of the final means than like increases in the successively smaller units, although this advantage is offset to some extent by the greater cost of increasing the number of samples in the larger areas. The mathematical relationship in this survey may be obtained from equation 5. The increased effectiveness of additional samples on the reliability of the means becomes less pronounced as the number of samples increases, owing to the fact that the reliability of estimates of a mean is directly in proportion to the number of samples. No one phase, therefore, is increased indefinitely without regard to the others, and a proper balance in sampling is maintained.

SUMMARY

The data used for the analysis of the sampling variability in this study consisted of the records of the 1931 and 1932 fall surveys, for the number of European corn borers per 100 plants, of a total of 66 county units located in the States of Indiana, Michigan, Ohio, Pennsylvania, and New York, infested by the one-generation strain. The methods of determining the sampling variability within the fields, for the estimation of the field means, and the variability from field to field within the county (part of which pertains to the reliability of the estimates of the mean level of population in the counties) were analyzed in considerable detail.

In sampling within the fields 4 units of 25 consecutive plants were first examined to determine the number of infested plants out of 100 plants; then from 5 to 10 of these infested plants were dissected to furnish an estimate of the average number of borers per infested plant. The product of these two means gave the estimates of the field mean number of borers per 100 plants. The equation for the estimation of the variance of products from the variance of the factors was used to obtain the estimates of the variance of these field mean numbers of borers per 100 plants. This method expressed the variability of sampling within the fields in terms that permitted the direct comparison of the contributions of any combination of the two sets of factors to the final variability of the estimate of the field mean.

The variability from field to field within the counties was divided into three main portions by the methods of the analysis of variance. The first portion consisted of the variability between county sections each of which was arbitrarily chosen to include one-fourth of the fields sampled in the county. This portion of the variability, which represented the estimated actual differences in level between the sections, is irrelevant to the estimate of the reliability of the county means, and illustrates the effect of applying the principle of local control in sampling in area-wide surveys of insect abundance.

The variability of field means from the respective section means is divided into two portions, one representing the estimates of the variability of the true field levels from the respective section levels, and the other representing the variability of the estimates of the field means for a given system of sampling within the fields.

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AN EXAMPLE OF THE ABILITY OF RIBES LACUSTRE TO INTENSIFY CRONARTIUM RIBICOLA ON PINUS MONTICOLA[1]

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INTRODUCTION

Four species of *Ribes*[3] are of special importance in the control of white-pine blister rust (*Cronartium ribicola* Fisch.) in the commercial range of western white pine (*Pinus monticola* Dougl.) in northern Idaho, northeastern Washington, and western Montana because of their abundance, distribution, and reaction to the rust (*12*).[4] These, in the order of their numerical prominence, are *Ribes lacustre* (Pers.) Poir., *R. viscosissimum* Pursh, *R. inerme* Rydb., and *R. petiolare* Dougl.

Extensive field studies in the rust-infected regions of western North America have shown that very wide differences exist among these four species both as to their susceptibility to the rust and to the subsequent development of telia (*9*).[5] Their order of prominence in these respects is: *Ribes petiolare*, *R. inerme*, *R. viscosissimum*, and *R. lacustre*, the first two species being relatively very high, the third medium to low, and the last very low. On the basis of the high susceptibility of and profuse telial development on *R. petiolare* and *R. inerme*, as observed early in the course of field studies in British Columbia (*3*), it was obvious that rapid and severe damage to the associated stands of western white pine (*Pinus monticola* Dougl.) would occur as the rust spread into the region centering in northern Idaho. Both *R. petiolare* and *R. inerme* are confined by habitat requirements to limited areas along stream courses, which are favorable sites for the development of the fungus. Therefore, in the region under discussion, the large-scale protection work against the rust carried on by the Bureau of Entomology and Plant Quarantine, United States Department of Agriculture, had as its immediate objective the systematic eradication of ribes in the stream-type areas where one or more of these highly susceptible species occurred (*13*).

As the rust spread and intensified in the region comprising northern

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[3] The genus name *Ribes*, as well as the common noun "ribes," is used in this paper to include both currants and gooseberries.

[4] Reference is made by number (italic) to Literature Cited p. 882.

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Idaho, western Montana, and northeastern Washington, continued observations showed that pine infection was developing in damaging proportions in upland areas where both *Ribes viscosissimum* and *R. lacustre* were present. Recognition of this condition made it necessary for the control program in this region to include the eradication of these species over the upland areas as well as the more susceptible species found only in the stream-type areas. Since, however, *R. lacustre* almost always occurs in association with other *Ribes* species, practically nothing was known regarding its pine-infecting capacity, but, on the basis of previous knowledge of its reaction to the rust, together with observations in the field, the species was thought to be relatively innocuous. The present study was undertaken to test this assumption.

This paper reports the results to date of a study made over a period of several years in the vicinity of Revelstoke, British Columbia, on a plot situated within the only known area in western North America where the prickly currant (*Ribes lacustre*) has been solely responsible for blister rust infection of a stand of western white pine. Because the Revelstoke area is well within the range of *Pinus monticola*, results of the study are considered of value in connection with control of the rust in the region centering in northern Idaho.

LOCATION AND DESCRIPTION OF THE STUDY PLOT

The plot is located in the interior of British Columbia, approximately 9 miles north of Revelstoke and on the east side of the Columbia River. It is 45 acres in size and is situated within the edge of an old burn on a steep mountainside with a westerly exposure. A few small creeks, the largest of which enters the plot near the southeast corner and runs out midway of the western boundary after forking several times, drain the area very well (fig. 1). A large rockslide near the northeast corner considerably reduces the density of vegetation in that part.

Bordering the plot on the south side is a stand of mature timber, composed mainly of western hemlock (*Tsuga heterophylla* (Raf.) Sarg.), western red cedar or giant arborvitae (*Thuja plicata* D. Don), and Douglas fir (*Pseudotsuga taxifolia* (Lam.) Britton), which extends southward for about 6 miles. To the north and east is an extensive burned area on which there occurs a sparsely stocked stand of reproduction composed mainly of western white pine and aspen (*Populus tremuloides* Michx.). Near the west boundary is the Columbia River, and on the opposite side of it there is a very extensive stand of mature timber composed mainly of western hemlock, western red cedar, and western white pine.

The only pine, and also the predominant tree species, on the plot is *Pinus monticola*. All species are unevenly distributed on the area, with the greatest density of growth on the southwest half. At first glance the stand appears to be almost pure pine in its composition, and in this regard it is typical of many others in the region. The principal tree associates of the pine are alder (*Alnus sinuata* (Regel) Rydb.), willows (*Salix* spp.), paper birch (*Betula papyrifera* var. *subcordata* (Rydb.) Sarg.), aspen, Douglas fir, western hemlock, and western red cedar. The stand is in the reproduction stage and was about 25 years of age at the time the study was started. The pines averaged slightly over 12 feet in height.

Bushes of *Ribes lacustre*, the only *Ribes* species present, are concentrated along the creeks and scattered elsewhere on the plot (fig. 1). This same species was also noted in moderate abundance along streams and on moist areas on the lands surrounding the plot, particularly to the south, east, and west. No other *Ribes* species were found closer than about 8 miles distant.

FIELD PROCEDURE

The area was located in the late fall of 1927, but no work was done on it that year. In 1928 the plot boundaries were surveyed and the 45-acre area within them was mapped and subdivided into blocks 1 chain square (fig. 1). Numbered stakes were placed at all block corners to facilitate future work.

In the summer of 1929, all the *Pinus monticola* trees on the plot were numbered, and their positions, together with the positions of all *Ribes lacustre* bushes, were mapped. Also, the number of feet of live stem for each ribes bush was obtained for use in computing the average amount of live stem per acre. No general survey of pine infection was made, but all blister rust cankers observed during the course of the other work were tagged and significant data recorded. Systematic examinations were made, however, of 100 bushes of *R. lacustre* to determine the amount of rust present on them. These bushes, which mainly occurred scattered over that portion of the plot having the greatest density of

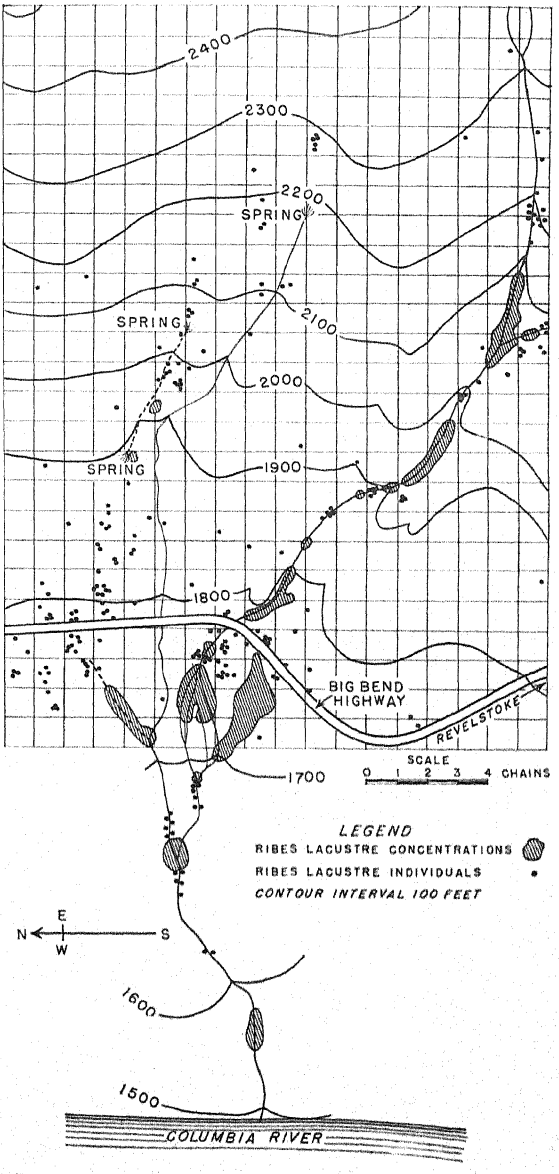


FIGURE 1.—Revelstoke natural pine infection area plot, Revelstoke, British Columbia.

the ribes and the pines, were tagged and used in subsequent years for the purpose of measuring annual infection. On the basis of the author's past experience with the rust, the 100 bushes were considered more than an adequate number for giving an index of the degree of infection over the entire plot. Also, extensive observations indicated that the infection on them was as great, if not slightly greater, in amount than would be the average for all bushes.

The systematic examination of the pines for blister rust infections was started in mid-June 1930. A numbered tag was attached to each canker found in order to prevent duplication and for future reference. Recorded data included: (1) Location on the tree of the infected branch, (2) diameter of the branch at the lower limits of the canker, (3) distance from the lower limits of the canker to the bole, (4) diameter of the bole at junction of the infected branch, (5) age of growth on which the canker originated, and (6) stage of development of the canker. Using these data, together with information on canker growth and effects reported by Lachmund (*6*), experienced assistants made estimates in the field as to whether the fungus would grow down the branch and into the bole. In the event that this was expected to happen, the number of years required for such growth and for the consequent death of or severe damage to the tree was also estimated. Limited time and personnel permitted examination of approximately only 60 percent of the trees in 1930, the remainder of them being examined during the following season.

The data available by the fall of 1931 showed that practically all pine infection on the plot occurred during the years 1922 to 1928, inclusive, the greatest part of it having originated in 1927. Any infection that might have taken place subsequent to 1928 was not yet readily apparent. Observations made in the fall of 1931 and spring of 1932, however, revealed that numerous cankers had not been found during the course of the examination in 1930, apparently mainly because of their small size at that time. Consequently, all the pines were reexamined in the summer of 1932, at which time many additional cankers of 1927 origin and some of 1928 origin were found. All of these were tagged and the usual data on them were obtained and recorded. Since the reexamination was being made 5 years after the heavy wave of infection in 1927 and 4 years after a much lighter wave in 1928, it was concluded, on the basis of earlier studies on canker growth and development (*4*, *5*, *6*), that all cankers having their inception in those years and previously should be of sufficient size to be readily visible in 1932. A check made a few years later indicated that this conclusion was correct. However, it was also found that a small percentage of cankers had still escaped detection during the reexamination. Failure to find these few was not ascribed to their small size but to the human factor involved.

RIBES INFECTION

No records of the extent of blister rust on *Ribes lacustre* are available for 1922, the year of first-known pine infection, nor were systematic data obtained for 1927, the year of greatest intensification of the disease on pine. The first examination of *R. lacustre* was but a cursory inspection of about 30 bushes made in the fall of 1927 at the time the area was discovered. Less than half of them bore evidence of

the rust, which was present only in slight amount. In the fall of 1928 an intensive survey was made of this host on the plot, and again the rust was found to be very sparse, the degree of infection being less than half that recorded in any subsequent year. Starting in 1929, however, systematic data were obtained annually until 1935 on the 100 tagged bushes previously mentioned. These data, which are summarized in table 1, were recorded and computed by the same methods described in a previous paper (*9*).

From table 1 it is evident, even for those years in which there occurred the greatest development of the rust on *Ribes lacustre*, that while only an extremely small percentage of the total lower leaf area became infected and a still smaller percentage bore telia there has been an almost consistent annual increase in the amount of rust. From these records, together with those obtained in 1927 and 1928, it would seem possible that a similar relationship might have extended back to 1922. If this relationship did not extend back to 1922, it still is reasonably safe to assume that the fungus was never much more abundant on *R. lacustre* than in those years for which data are available, for, as is later shown herein, very few sporulating cankers could have been present on the area to have caused such infection. Even if the rust was more prevalent than that shown from 1929 to 1934, however, it seems doubtful if it ever was present in sufficient amount to be classed as a heavy attack on this *Ribes* species. In support of these suppositions it may be demonstrated that the recorded infection has been very light when percentages of total leaf area infected and total leaf area bearing telia (table 1) are compared with the average figures for susceptibility and telium-producing capacity of *R. lacustre* presented in table 2. The figures in table 2 were obtained by Mielke, Childs, and Lachmund in their large-scale relative-susceptibility studies (*9*). Since nearly all the *R. lacustre* bushes on the Revelstoke plot grew in the shade or partial shade of other vegetation, the data presented for them should be compared mainly with the data for corresponding growth forms shown in table 2.

TABLE 1.—*Annual blister rust infection of 100 tagged Ribes lacustre bushes on the Revelstoke plot*

Year	Bushes		Total leaves		Total lower leaf area	
	Examined	Infected	Examined	Infected	Infected	Bearing telia
	Number	*Number*	*Number*	*Number*	*Percent*	*Percent*
1929	100	39	76,585	738	0.05	0.01
1930	100	93	59,650	837	.07	.03
1931	100	100	56,710	1,815	.19	.05
1932	100	100	36,745	5,144	.59	.17
1933	100	100	57,415	6,316	.33	.07
1934	98	98	65,965	6,597	.35	.04

TABLE 2.—*Average figures for susceptibility and telium-producing capacity of Ribes lacustre*

Form of *R. lacustre* as related to exposure to sunlight	Total lower leaf area	
	Infected	Bearing telia
	Percent	*Percent*
Open	3.4	0.4
Part-shade	4.3	.7
Shade	8.1	2.9

PINE INFECTION

As a result of the examination of the pines made during the years 1929 to 1932, inclusive, a total of 10,109 cankers was recorded. Analysis of the data, by Lachmund's (*4*) method of determining the age of blister rust infection on western white pine, indicated that 337 of these cankers had their inception in 1922, 150 in 1924 and 1925, and the remainder, 9,622, in 1927 and 1928. Possible infections originating in 1929 or subsequently had not yet reached a readily visible stage of development at the time of the last examination in 1932, and consequently no particular effort was made to record them. Therefore, only those cankers that had their inception in the years 1922 to 1928, inclusive, are considered in the study.

Neither the exact place at which pine infection first became established on the area nor any original canker was discovered. As a result of extensive studies on the ground, however, it was evident that both the place of origin and the center of pine infection were well within the plot boundaries. Of the oldest cankers found 337 were on the plot and a few others in the immediate vicinity, all of which had their inception in 1922, but that year is not regarded as the one in which pine infection originally occurred on the area. According to the author's experience in the West, the abundance and scattered distribution of these cankers would strongly indicate that a few older ones must have been present at one time, for almost invariably the number of trees originally infected and the number of original cankers at an infection center are comparatively small, being generally less than 10 even when highly susceptible ribes species are present. In this case, over 300 widely distributed pines bore the cankers that originated in 1922, and consequently it would seem reasonable to assume that some pine infection must have occurred prior to that time. Considering the available evidence, together with the normal development of the fungus (*5*) and the general spread in 1917 and 1918 (*3*), it is believed that the rust first became established in pines on the plot in either one of those years. Failure to find any older cankers may very possibly be attributed to destruction of the tree or trees that bore them when a highway (fig. 1) was built through the area about 1925, for this highway crosses the part of the plot that has the greatest density of pine infections.

The results of the studies on pines are summarized in table 3. Observations made in 1933 indicated that possibly as many as 5 percent or more of the cankers had been missed during the examinations, consequently the number of trees infected, the number of cankers, and the degree of damage were somewhat greater than the corresponding data shown in the table. Since, as previously stated in the section on Field Procedure, the data on tree damage are based mainly on estimates of future development of the fungus in the bark, their reliability may possibly be questioned. All cankers found were tagged, however, and it was therefore feasible to make a future check of the estimates. This was done in the fall of 1936, five to seven seasons later, depending upon the time the original work was done. Results of the check indicated that the estimates were of sufficient accuracy to make revision of the summary data unnecessary. From the data in table 3, it is anticipated that a higher percentage of trees infected in 1922 will be killed or damaged than of the trees infected during the entire period of 1922–28. A discussion of the factors

responsible for this percentage difference is not considered within the province of this paper, but they are known to include height of tree, width of crown, and difference in basis of trees infected in the different years.

TABLE 3.—*Blister rust on western white pine on the Revelstroke plot*

Years in which infection occurred	Trees						Cankers		
	Total on plot	Infected		To be killed or damaged [1]			Total	Average per tree on basis of—	
				Number	Percentage of infected trees	Percentage of total trees		Infected trees	Total trees
	Number	*Number*	*Percent*				*Number*	*Number*	*Number*
1922	9,316	312	3.35	203	65.06	2.18	337	1.08	0.036
1922 to 1928, inclusive	[2] 9,920	3,417	34.45	1,600	46.82	16.13	10,109	2.96	1.02

[1] The term "damage", as used in this paper, denotes injury to the pines to the extent of preventing ultimate merchantable value.
[2] The difference in total number of trees is accounted for by the increase in number of seedlings.

Although it is evident that the intensification up to 1928, inclusive, has been very rapid, this cannot be considered as exceptional, for instances of similar and even greater rates of increase in the rust on *Pinus monticola* have been reported (*7; 8, p. 303; 11*), and the author has observed many others during the past 12 years. If the rust continues to increase at the same rate that it did from 1922 to 1928 (table 3), nearly all pines on the plot will be infected by approximately 1940. An examination of the weather records for Revelstoke indicated that both 1922 and 1927 were favorable years for the infection of pines, whereas the intervening years and 1928 were relatively unfavorable. Approximately only one season in every 5 years has been favorable for general spread of the rust in the interior region, while in the coastal region of British Columbia, Pennington (*10*) found this average to be one in every 4 years. Accordingly, it would appear that intensification of the fungus on the plot will be governed largely by climatic conditions and will occur at an irregular rate. A reliable estimate of the time required for infection of all pines is therefore difficult to make.

The number of feet of live stem per acre is commonly used as a measure of the abundance of a *Ribes* species on an area. It seemed desirable, therefore, to use this criterion in determining the amount of *Ribes lacustre* on the plot. As the result of a careful check, a total of 24,111 feet, or an average of 536 feet per acre, was found. The bushes, which varied greatly in size, were not generally distributed over the area, but occurred in greatest density in the vicinity of the creeks (fig. 1). *R. lacustre* was also found in the immediate vicinity of the plot, but in lesser densities, and because of their location these bushes are not regarded as being responsible for many of the pine infections. Whether occurring on or off the plot, however, *R. lacustre* was solely involved, for it is the only species of *Ribes* known for several miles around. There are plantings of the cultivated black currant (*R. nigrum* L.) about 8 miles distant, but spores from these plants are not believed to have caused any of the cankers on the plot, for no instances

are known of spread from *Ribes* to pines over distances even one-third as great.

The pines occur in greatest density on the south half of the plot, and except for a few small areas the stand cannot be considered as fully stocked, for in 1927 there was an average of only about 220 trees per acre. The older trees have been bearing cones for several years, and some seedlings appear annually. However, if the present rate of intensification of the rust and the small number of cankers required to kill such young trees are considered, few of them may be expected to survive for very long. Accordingly, there appears no possibility of the area becoming fully stocked with white pine or of the present stand reaching maturity so long as *Ribes lacustre* is present.

DISCUSSION

Western white pine occurs in two separate belts, which may be designated as coastal and interior. Both the Revelstoke area in British Columbia and the region centering in northern Idaho are within the interior belt. The same tree species occur over most of this region, and the summer climate is fairly uniform, although a somewhat greater amount of precipitation occurs at Revelstoke than in northern Idaho. This similarity in general conditions between the two places would seem to make the results of the study of more than local application. Before any final conclusions are reached, however, certain climatic factors should be more carefully considered, for weather conditions to a large extent determine the spread and severity of blister rust. According to Pennington (*10*), moisture is more important than temperature for infection of pines and ribes. Because of the importance of moisture, a comparative summary of the rainfall during the growing season at Revelstoke, the weather station nearest the plot, and various places in north Idaho is given in figure 2. It is evident from the pine-infection data that *Ribes lacustre* caused rapid intensification of the rust on *Pinus monticola* near Revelstoke, but rainfall there is somewhat more abundant than at the weather stations in Idaho used for comparison. This difference in rainfall is apparently of little consequence, however, for the author has been unable to detect its effect in any difference in the development of the rust between the two regions, and according to various reports (*1*, *2*, *11*, *12*, *13*, *14*), the fungus is well established and intensifying at a very rapid rate in the white pine forest of Idaho. The results of the study would therefore appear to be of value in connection with control of the rust in the region centering in northern Idaho.

The rapid intensification of the rust that occurred on the plot was contrary to expectation, for it was thought that *Ribes lacustre*, with its low susceptibility and low telium-producing capacity, would not cause such abundant infection of pines. The data strongly indicate, however, that *R. lacustre* constitutes a far greater menace than has hitherto been assumed and that susceptibility and capacity to produce telia are not necessarily safe indexes of the relative importance of a *Ribes* species as a source for pine infection. The results of the study are of special significance in illustrating that stands of western white pine in infected areas may be severely damaged by blister rust if *R. lacustre*, which is the least susceptible of the four most important species in the pine region centering in northern Idaho, is allowed to remain in considerable numbers within and adjacent to the stands.

Aside from the fact that *Ribes lacustre* is capable of rapidly intensifying blister rust on western white pine, there are other factors that make it a very important host in the region centering in northern Idaho. Numerically it is the most abundant of the four common ribes in that region, as evidenced by the control work from 1923 to 1935, inclusive, for nearly one-half the total number of ribes destroyed were of that species.[6] *R. lacustre* is widely distributed over the region, occurring generally in the forested area. It is a tolerant species and is not confined to any particular site conditions, growing not only in moist soils bordering streams but also on the drier upper slopes. The wholesale destruction of this plant by chemical means has not yet been practicable, partly because of its resistance to ordinary dosages of chemicals that may be safely or economically used. Since *R. lacustre* is a species that propagates readily by layering, hand eradication is somewhat more difficult than with some of the other ribes. It would seem, therefore, that *R. lacustre* is as much if not more of a problem in the control of the rust than some of the more susceptible species.

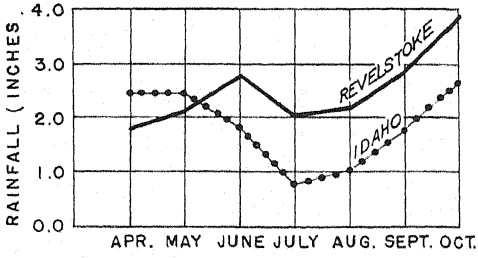


FIGURE 2.—Graphical summary of rainfall at Revelstoke, British Columbia, and in Idaho. The monthly rainfall averages for Revelstoke cover a period of 32 years and were taken from British Columbia Department of Agriculture weather records, Report for 1933. The averages for Idaho were computed from United States Weather Bureau records obtained at Avery, Prichard, St. Maries, Priest River Experiment Station, and Pierce, covering various periods ranging from 16 to 32 years.

SUMMARY

Four species of *Ribes* are of special importance in the control of white-pine blister rust (*Cronartium ribicola*) in the western white pine region centering in northern Idaho. Numerically, the most prominent of these is the prickly currant (*Ribes lacustre*), which is relatively very low both in susceptibility to the rust and in the production of telia. This paper reports the results of a study, conducted over a number of years on a 45-acre plot near Revelstoke, British Columbia, on the ability of this currant to spread blister rust to western white pine (*Pinus monticola*).

Although the known infection of *Ribes lacustre* on the area has been relatively small in amount for this species, the rust has intensified rapidly on the pines. The available evidence indicates that a few trees first became infected in either 1917 or 1918. In 1922 over 3 percent were infected and by the end of 1928 about 34½ percent of the total of 9,920 pines on the plot were diseased. The cankers increased in number from 337 in 1922 to 10,109 in 1928. This attack has been severe enough to result in the death or serious damage of 1,600 trees, or over 16 percent of the total.

If a similar rate of intensification of the rust continues, it will obviously be but a matter of a few years before practically all the pines become infected and serious damage or death results. There appears no possibility that the stand of western white pine on the area will reach maturity under the prevailing rust conditions.

Results of the study indicate that *Ribes lacustre* constitutes a far greater menace to western white pine than has hitherto been assumed

[6] Information furnished by officials of the Division of Plant Disease Control, Bureau of Entomology and Plant Quarantine, and used here with their permission.

and that this species must be eradicated where it occurs in or near pine stands if such stands are to be protected against the rust.

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WATER SOAKING OF LEAVES IN RELATION TO DEVELOPMENT OF THE BLACKFIRE DISEASE OF TOBACCO[1]

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INTRODUCTION

In a previous publication [2] the general situation with respect to the wildfire and the blackfire or angular leaf spot diseases of tobacco (*Nicotiana tabacum* L.) was discussed and the need for explaining the occurrence of leaf spot epidemics was indicated. It was shown that epidemics of wildfire (*Bacterium tabacum* Wolf and Foster) are conditional on the occurrence of leaf water soaking, and the factors modifying leaf resistance to water soaking were discussed. These results are extended in the present paper by similar studies with the blackfire (*Bact. angulatum* Fromme and Murray) disease.

Blackfire is the common tobacco leaf spot disease of Virginia, North Carolina, Tennessee, Kentucky, and Wisconsin. Following its identification by Fromme and Murray,[3] it was long regarded as established that the disease was caused by *Bacterium angulatum* and was serious and destructive. It was a fact, however, that no one had ever produced the "epidemic" type of blackfire (see fig. 2, *A*) under controlled conditions. Inoculations produced only small localized lesions (fig. 1), and in consequence Valleau [4] suggested that the epidemic type of leaf spot was nonparasitic in nature. It is believed that the results presented in this paper adequately clear up this situation.

EXPERIMENTAL RESULTS

Methods of study and preliminary results with *Bacterium angulatum* paralleled in all details those given in a previous publication [5] for *Bact. tabacum*.

In repeated controlled experiments, it was demonstrated that the difference between disease development as shown in figure 1 and figure 2, *A*, was entirely dependent on leaf water soaking (fig. 3). Thus, for example, 12 leaves were selected and one-half of each was sprayed until a water-soaked condition of these areas was produced. Sixty-five of the water-soaked areas were then inoculated by pricking them lightly with a needle dipped in a suspension of the bacteria, and an equal number of inoculations were made on the unsprayed halves. The remaining water-soaked areas were left uninoculated. The inoculations of water-soaked areas produced lesions in every case, ranging

[1] Received for publication Aug. 18, 1937; issued February 1938.

[2] CLAYTON, E. E. WATER-SOAKING OF LEAVES IN RELATION TO DEVELOPMENT OF THE WILDFIRE DISEASE OF TOBACCO. Jour. Agr. Research 52: 239-269, illus. 1936.

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[4] VALLEAU, W. D. ARE BLACKFIRE AND ANGULAR LEAF SPOT OF TOBACCO IDENTICAL? (Abstract) Phytopathology 19: 93. 1929.

[5] CLAYTON, E. E. See footnote 2.

from ⅜ to 1⅞ inches in diameter. The inoculations without water soaking produced either no infection or at most a lesion less than one-eighth of an inch in diameter. The water-soaked condition of areas that were not inoculated disappeared without any leaf injury. Furthermore, the lesions on the water-soaked areas developed so rapidly that they were of large size when the plants were removed from the damp chambers (60 hours after inoculation).

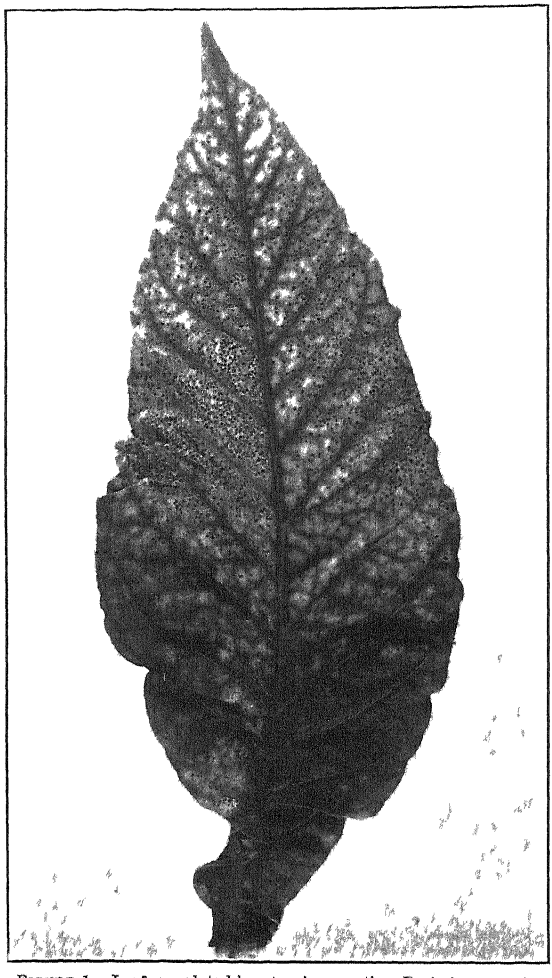


FIGURE 1.—Leaf inoculated by atomizing with a *Bacterium angulatum* suspension, photographed 20 days later The lesions have attained full size and are typical of those obtained without the aid of water soaking

INOCULATION OF UPPER AND LOWER LEAF SURFACE

In the work with wildfire it was found that, provided water soaking was present, there was little or no difference in the results from lower or upper leaf-surface inoculation. With *Bacterium angulatum*, which appears to be a less virulent parasite, there were significant differences in results between the two surfaces (table 1).

TABLE 1.—*Effects of different combinations of water-spray and inoculation treatments on the upper and lower leaf surfaces*

Leaf no	Leaf surface given—		Initial lesions	Leaf area finally killed	Leaf no	Leaf surface given—		Initial lesions	Leaf area finally killed
	Water spray	Inoculation				Water spray	Inoculation		
			Number	*Percent*				*Number*	*Percent*
1	Upper	Upper	36	20	1	Lower	Upper	125	20
2	do	do	36	50	2	do	do	13	10
3	do	do	20	20	3	do	do	70	10
1	do	Lower	250	80	1	do	Lower	224	40
2	do	do	154	50	2	do	do	168	20
3	do	do	150	100	3	do	do	450	50

Thus, with *Bacterium angulatum*, even with water soaking, the number of initial lesions and usually the subsequent disease damage were much greater from inoculation of lower leaf surfaces, though it did not appear to matter greatly whether the water soaking was produced by spraying upper or lower surfaces. The check inoculations of leaves not soaked that accompanied this experiment produced only scattering small lesions, and these caused practically no leaf damage.

EFFECT OF SIZE AND PERSISTENCE OF WATER-SOAKED AREAS ON LESION DEVELOPMENT

Experiments with size of water-soaked areas in relation to blackfire development showed that, as with wildfire, larger water-soaked areas

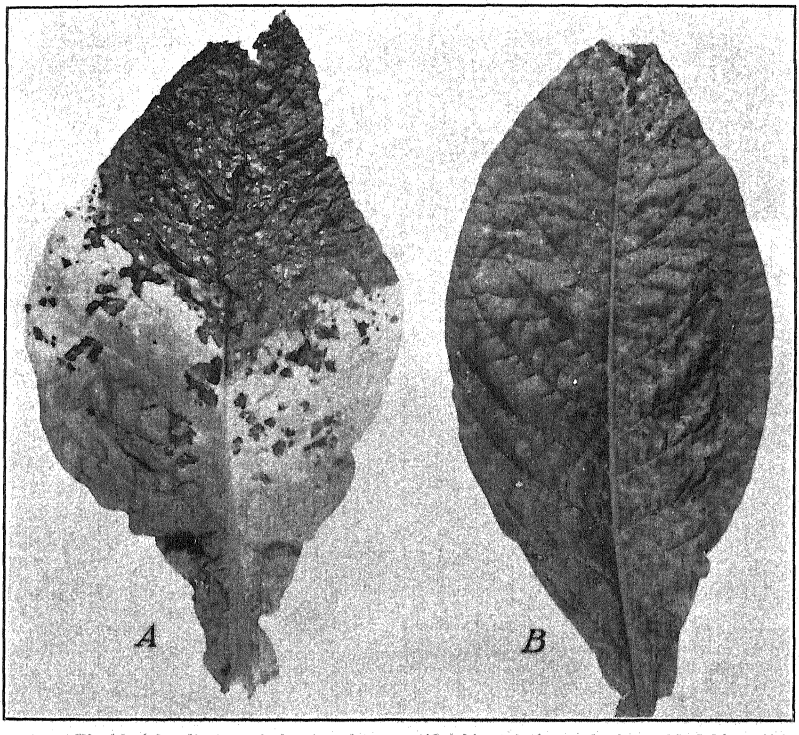


FIGURE 2.—The blackfire disease as it developed from artificial inoculation under favorable field conditions: *A*, Lower leaf, showing typical symptoms of the destructive epidemic type of disease which is usually called blackfire; *B*, upper leaf from the same plant, showing lesions of a type frequently described as angular leaf spot.

favored the development of larger lesions. Thus, the following results were obtained:

Leaves not water-soaked.—Twenty prick inoculations gave either no infection or a mere trace.

Leaves water-soaked, areas one-eighth to three-eights of an inch in diameter.—20 prick inoculations gave lesions ranging from one-eighth to three-eighths of an inch in diameter.

Leaves water-soaked, areas ¾ to 1½ inches in diameter.—Lesions ranged from one-fourth to three-fourths of an inch in diameter.

With small areas, the average size of the lesions was 0.3 inch, which was about the average size of the original water-soaked areas. The

lesions with largew ater-soaked areas averaged 0.7 inch in diameter, but this was much less than the size of the original water-soaked areas.

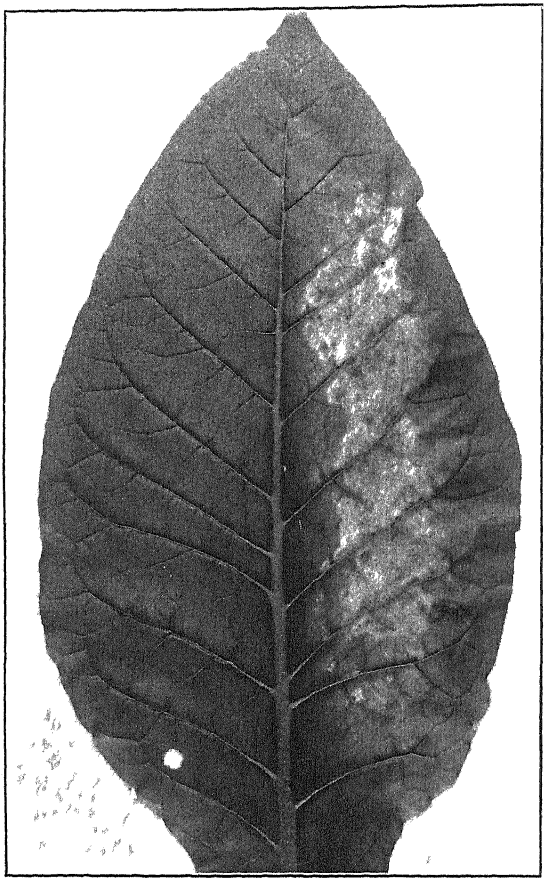


FIGURE 3.—Entire leaf inoculated with a *Bacterium angulatum* suspension. Right half previously water-sprayed, left half not water-sprayed.

Repeated tests have shown that this is the usual condition, namely, that small water-soaked areas are completely invaded but larger areas are not.

The time that the water-soaked leaf condition persists after infection has occurred is of great importance in blackfire development, as is shown in table 2 and figures 4 and 5.

The actual number of infections secured was about the same when the leaves were water-soaked for 10, 24, or 50 hours, but with the shorter periods development of the lesions was cut short and damage to the plant was slight. Figure 4, *A*, shows that it is quite possible for leaves to be freely infected by *Bacterium angulatum* and then, even after epidemic disease development is well under way, the progress of the disease can be abruptly checked. These results explain why even severe storms, when followed at once by clear weather, are not effective in producing blackfire outbreaks.

TABLE 2.—*Relation of persistence of water-soaked areas to disease development*

Leaf no	Leaf area killed after 10 days when water soaking was continued for—			Leaf no.	Leaf area killed after 10 days when water soaking was continued for—		
	10 hours	24 hours	50 hours		10 hours	24 hours	50 hours
	Percent	*Percent*	*Percent*		*Percent*	*Percent*	*Percent*
1	1	3	20	8	2	3	20
2	1	8	15	9	1	5	75
3	1	5	15	10	2	10	60
4	1	5	25	11	2	8	75
5	2	3	25	12	2	8	75
6	1	5	25				
7	6	2	15	Average	1.8	5.4	57.1

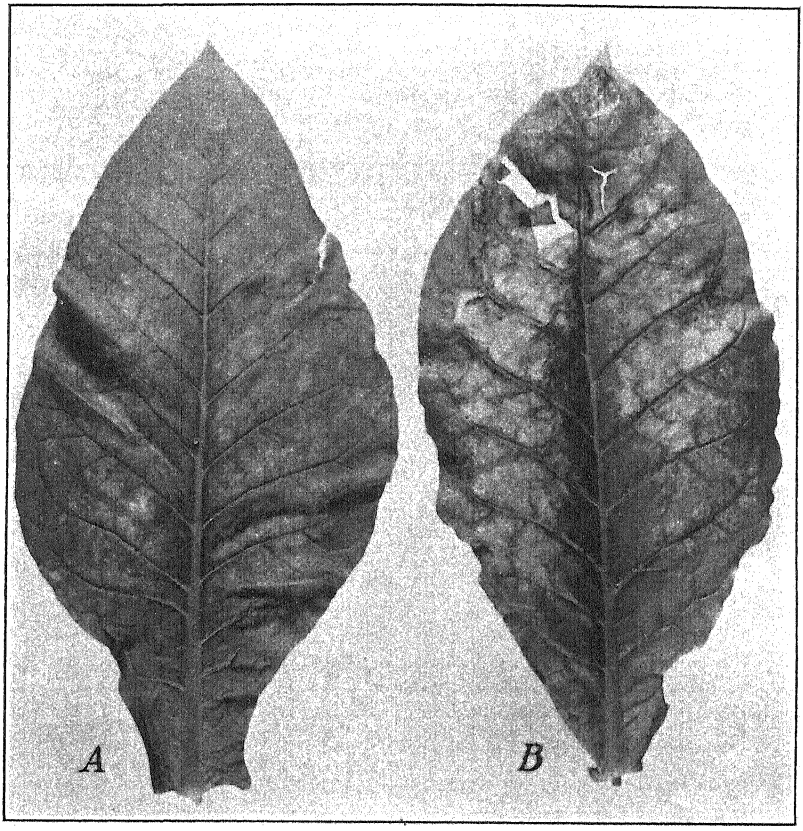


FIGURE 4.—Effect of duration of water-soaked condition on disease development. Both leaves were water-soaked and inoculated alike. With *A* the water-soaked condition was maintained in a saturated atmosphere for 24 hours; with *B*, for 50 hours. Note the heavy infection but arrested disease development on *A*; with *B*, the final result was extensive tissue destruction.

EFFECT OF TOPPING AND FERTILIZATION PRACTICES ON SUSCEPTIBILITY TO BLACKFIRE

Height of topping has a marked effect on the susceptibility of the host plant to epidemic blackfire, just as was previously shown for wildfire. In table 3 results are given from a field test conducted in 1935. All plots were uniformly inoculated and conditions were moderately favorable for disease development. Low-topped plants averaged 10 leaves per plant; high-topped plants, 18 leaves; plants not topped, 25 leaves. The percentage of leaf area destroyed by disease was estimated for 40 leaves from each plot.

Topping effects are at once marked and consistent, and low topping is undoubtedly a major factor in promoting blackfire damage in the dark-fired tobacco-producing areas where this practice is essential to produce a large, heavy type of leaf.

It was not possible to make so detailed a study of fertilizer influences on blackfire development as was made on wildfire development. However, numerous observations indicate clearly that low-potash and high-nitrogen fertilization increase blackfire damage. These fertilizer effects were very apparent in plot work conducted on soils of

low natural fertility, where the same fertilization treatments were repeated year after year; but on moderately fertile soils, with the application made but a single year, even marked variations in the quantities of nitrogen and potash applied in the fertilizer had but little effect on blackfire development.

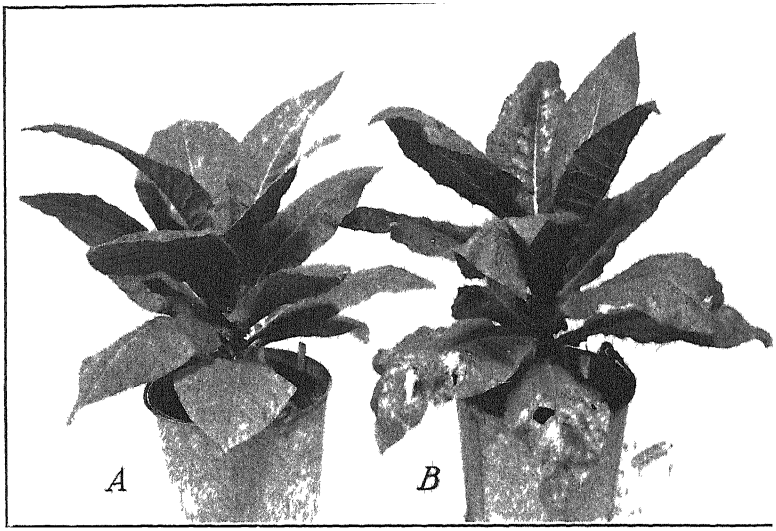


FIGURE 5.—Effect of water soaking and duration of water-soaked condition on blackfire development for entire plants. Both plants were water-soaked and inoculated alike. *A* was then held in a saturated atmosphere for 10 hours; *B*, for 50 hours. Photographed 12 days later.

TABLE 3.—*Percentages of leaf area killed following different heights of topping*

Low topping [1]	High topping [1]	No topping [1]
60–30– 8–20–15	2– 5–30– 5–30	8– 2– 5– 2– 2
5–12–20–15– 5	10–10–10– 6–10	5– 1– 5– 1– 4
2– 4– 6–10–20	2– 5– 3–15– 5	4– 1– 2 2– 3
10–12– 4–50–10	10– 5– 4– 8– 6	5– 5 2 5 2
30–10–20– 8–30	8– 3–10–10–10	3 5– 2– 8–25
25–15– 5–10– 2	5– 8– 8– 2– 8	4– 5–20–40– 1
20–12– 5–35–30	8–40– 2– 8– 8	2– 5– 3 4– 5
20–25–20–30– 2	5–30– 8– 4–15	.5– 1– 2– 2 25
Average 16.8	Average 9.5	Average 5.4

[1] Each of the following 40 percentages refers to a separate leaf. The values are arranged in groups of 5 merely for convenience and to save space. No relationship exists among values occupying similar positions in different columns.

DISCUSSION

The results secured in these experiments with blackfire, caused by *Bacterium angulatum*, are similar in every respect to those previously reported for wildfire, and it seems clear that with types of tobacco grown in the United States epidemic development of blackfire is dependent on water soaking of the leaves. Water soaking breaks down host resistance and permits successful and rapid tissue invasion by the organism.

Bacterium angulatum, while similar in mode of action to *Bact. tabacum*, is evidently a less virulent parasite. Thus, throughout the work

in parallel experiments, infection with *Bact. angulatum* in the absence of water soaking was less easy to secure and the infections were less numerous, developed more slowly, and were smaller; with water soaking and more favorable conditions, these differences still persisted though they were not so marked. With favorable conditions, both organisms can practically destroy a crop in a short time.

Some degree of blackfire protection can be secured by high topping, and low-nitrogen and high-potash fertilization is also helpful, but these practices can be applied only in areas where the type of tobacco grown will permit. They are applicable to the flue-cured area.

However, it is evident that effective blackfire control must be sought by other means, and the most promising of these are (1) sanitation and other measures designed to eliminate sources of infection and (2) development of varieties still more resistant to the disease than those now available. In the latter connection it is to be noted that varieties grown in the United States are practically all moderately resistant to blackfire, which accounts for the fact that they suffer little damage from the disease until this resistance is broken down by the conditions incident to heavy storms.

SUMMARY

Tobacco leaves are readily infected by *Bacterium angulatum*, but under ordinary conditions invasion is limited to small areas. The lesions are usually one-eighth of an inch or less in diameter, and large numbers of infections cause but little damage to most types of tobacco.

It had been suggested that the large, quickly developing lesions characteristic of epidemic blackfire were nonparasitic in nature; but it is now shown that they are caused by *Bact. angulatum*, but only under special conditions. The resistance of the leaf to invasion must first be broken down by water soaking, which in turn results from severe storms.

Even after leaves are water soaked, however, and infection has occurred, the development of the disease is abruptly checked if the water-soaked condition disappears within a few hours. Epidemic disease development was repeatedly obtained by water soaking the leaves for 48 hours. Resistance of the leaves to water soaking, and hence to the disease, has been shown to be greatly modified by topping and fertilization practices. High topping and low-nitrogen and high-potash fertilization increase leaf resistance to water soaking, and these measures are recommended as practicable in the flue-cured tobacco area.

43914—38——2

ROLE OF MOLYBDENUM IN THE UTILIZATION OF AMMONIUM AND NITRATE NITROGEN BY ASPERGILLUS NIGER [1]

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INTRODUCTION

A study of the factors associated with the very poor growth and sporulation of *Aspergillus niger* Van Tiegh under certain conditions of molybdenum deficiency, briefly mentioned in a former publication (*16*),[2] is reported in this paper. The evidence obtained renders it quite probable that one of the functions of molybdenum in plants is in the reduction processes whereby ammonium nitrogen is formed from nitrate nitrogen; that is, the marked effects of molybdenum deficiency appear to be associated with the presence and utilization of nitrate nitrogen. Its action seems to be unique in this regard, inasmuch as acidity, iron, zinc, copper, and manganese do not act similarly.

The data of prior investigations on the utilization of ammonium and nitrate nitrogen by green plants (*1*, *2*, *10*, *11*) and by fungi (*12*, *13*) have been explained variously on the basis of hydrogen-ion concentration and of iron, copper, and manganese concentration. The consensus of opinion is that the processes of nitrate utilization are quite similar in green plants and fungi (*4*) and culminate in the formation of ammonium ion for the synthesis of amino acid and protein. Certain differences exist, nevertheless. Green plants would appear to thrive best with nitrate nitrogen as a rule, though ammonium nitrogen can give results fully as good if the environmental factors are properly adjusted. Fungi, however, would appear to be more tolerant of variations in concentration of ammonium ion and other components of the nutrient solution. Moreover, certain genera of fungi have been found unable to utilize nitrate nitrogen (*7*, *9*). Experimental emphasis in the data on nitrogen utilization here reported has been placed on the maximum yield obtainable at a fixed level of 665 mg of nitrogen per liter. This is a minimum value and corresponds to that employed in the optimum solution (dibasic) containing 1.90 g of ammonium nitrate (NH_4NO_3) per liter (*16*). This procedure is based on the assumption that equal growth with two different forms of nitrogen under conditions equal or optimum in all other respects implies that the rate and extent of utilization of each are identical and therefore that no intrinsic difference in availability exists.

METHODS

The organism used in these experiments was *Aspergillus niger* ("W" strain). It was grown at 35° C. in 50-cc portions of a 5-percent sugar solution containing all necessary salts. Water redistilled in

[1] Received for publication July 2, 1937; issued February 1938.

[2] Reference is made by number (italic) to Literature Cited, p. 901.

pyrex glassware, sucrose containing not more than 0.0025 percent of ash, and reagent chemicals were used throughout. The mycelial felts were harvested, dried at 103°, and weighed as usual. Acidities were determined with a quinhydrone electrode. Earlier papers by the writer (*14*, *15*, *16*) should be consulted for additional details.

NUTRIENT VALUE OF VARIOUS FORMS OF NITROGEN

The experimental data of tables 1 to 3, inclusive, form a series designed to test four different nitrogen sources as regards their suitability for growth and their effect on the trace-element requirements of *Aspergillus niger*. The solution used in the experiments of table 1 is the dibasic optimum solution (*16*), except that sodium nitrate was tested also with a solution in which monopotassium phosphate was substituted for the dipotassium phosphate of the optimum solution. Equivalent quantities of ammonium nitrate, ammonium chloride, sodium nitrate, and urea, at a nitrogen level of 665 mg per liter, were employed. The experiments of table 2 are a repetition of those of table 1, except that each trace element (iron, zinc, copper, manganese, and molybdenum) has been increased where necessary to the minimum concentration that is optimum for growth. These experiments are again duplicated in table 3, but this time each trace element is present in minimum optimum quantity and the potassium phosphate and magnesium sulphate have each been increased 40 percent.

TABLE 1.—*Effects of different nitrogen compounds (665 mg of N per liter) in an optimum solution*[1] *(dibasic) on the growth of Aspergillus niger at 35° C. for 5 days, when heavy-metal concentrations optimum for NH_4NO_3 were employed*

Element omitted	NH_4NO_3				NH_4Cl				Urea			
	Yield per 2.5 g sucrose	Proportion of maximum yield obtained[3]	Acidity at harvest	Sporulation[4]	Yield per 2.5 g sucrose	Proportion of maximum yield obtained	Acidity at harvest	Sporulation	Yield per 2.5 g sucrose	Proportion of maximum yield obtained	Acidity at harvest	Sporulation
	Milligrams	*Percent*	*pH*		*Milligrams*	*Percent*	*pH*		*Milligrams*	*Percent*	*pH*	
Fe	236.1	21.14	1.90	5,bl	502.6	49.90	1.59	3,j	214.8	31.62	2.47	4,j
Zn	52.5	4.68	2.60	4,bl	90.5	9.39	2.27	4,j	42.6	6.27	2.69	2,j
Cu	991.7	88.48	2.81	2,y	918.3	91.16	1.38	1,w	616.9	90.82	2.51	4,t
Mn	766.0	68.38	1.67	2,bl	726.3	72.11	1.37	2,j	737.8	108.92	2.17	4,bl
Mo	800.4	71.42	1.73	3,bl	1,025.6	101.82	1.36	2,j	724.0	106.59	2.24	8,bl
None[4]	1,120.8	100.00	2.64	6,bl	1,007.3	100.00	1.35	2,j	679.2	100.00	2.33	10,bl
Maximum	[6] 1,131.5				[6] 1,070.0				[6] 832.4			
C. U.		[7] 45.26				[7] 42.80				[7] 33.30		
pH			[8] 7.14				[8] 7.01				[8] 6.98	

See footnotes at end of table.

TABLE 1.—*Effects of different nitrogen compounds (665 mg of N per liter) in an optimum solution* [1] *(dibasic) on the growth of Aspergillus niger at 35° C. for 5 days, when heavy-metal concentrations optimum for NH_4NO_3 were employed*—Continued

Element omitted	$NaNO_3$				$NaNO_3$ [2]			
	Yield per 2.5 g sucrose	Proportion of maximum yield obtained	Acidity at harvest	Sporulation	Yield per 2.5 g sucrose	Proportion of maximum yield obtained	Acidity at harvest	Sporulation
	Milligrams	*Percent*	*pH*		*Milligrams*	*Percent*	*pH*	
Fe	144.3	20.37	3.28	4,j	282.2	29.10	3.20	8,bl
Zn	31.6	4.45	3.06	2,j	61.3	6.32	2.99	3,j
Cu	698.0	98.53	2.75	2,t	1,013.8	104.53	2.96	6,t
Mn	655.6	92.54	1.89	2,j	937.2	96.63	2.05	1,j
Mo	73.4	10.35	3.03	3,j	83.0	8.55	2.69	3,j
None [5]	708.5	100.00	2.99	8,bl	969.9	100.00	2.97	10,bl
Maximum	[6] 748.5				[6] 1,035.4			
C. U		[7] 29.94				[7] 41.42		
pH			[8] 7.45				[8] 4.91	

[1] Water, 1,000 cc; sucrose, 50 g; NH_4NO_3, 1.90 g; K_2HPO_4, 0.35 g; and $MgSO_4.7H_2O$, 0.25 g.
[2] Used 0.55 g of KH_2PO_4 instead of 0.35 g of K_2HPO_4.
[3] Yield from complete medium, with none of the trace elements omitted, taken as 100 percent.
[4] Spore color is indicated by the initial letter or letters of the terms "jet," "black," "brown," "tan," "yellow," or "white," and extent of sporulation by the numerals 0 (no spores) to 10 (entirely covered with spores).
[5] Trace-element concentrations (milligrams per liter) were as follows: Fe, 0.20; Zn, 0.18; Cu, 0.04; Mn, 0.02; and Mo, 0.02.
[6] Maximum individual yield.
[7] Coefficient of utilization, or yield per 100 g of sucrose.
[8] Initial pH of nutrient solution.

TABLE 2.—*Effects of different nitrogen compounds (665 mg of N per liter) in an optimum solution* [1] *(dibasic) on the growth of Aspergillus niger at 35° C. for 4 days, when optimum heavy-metal concentrations were employed*

Element omitted	NH_4NO_3				NH_4Cl				Urea			
	Yield per 2.5 g sucrose	Proportion of maximum yield obtained [3]	Acidity at harvest	Sporulation [4]	Yield per 2.5 g sucrose	Proportion of maximum yield obtained	Acidity at harvest	Sporulation	Yield per 2.5 g sucrose	Proportion of maximum yield obtained	Acidity at harvest	Sporulation
	Milligrams	*Percent*	*pH*		*Milligrams*	*Percent*	*pH*		*Milligrams*	*Percent*	*pH*	
Fe	234.3	22.45	2.00	3, bl	466.6	48.03	1.67	4, bl	181.6	20.15	2.27	2, bl
Zn	118.6	11.36	2.41	4, bl	43.2	4.45	1.93	4, bl	100.2	11.11	2.71	2, bl
Cu	965.6	92.51	2.38	1, w	915.3	94.22	1.44	0	821.4	91.10	2.68	4, t
Mn	742.0	71.09	1.58	1, w	749.4	77.14	1.35	1, bl	784.6	87.03	1.96	4, bl
Mo	690.5	66.16	1.65	2, bl	974.3	100.30	1.42	3, bl	908.8	100.80	2.10	10, bl
None [5]	1,043.8	100.0	1.96	8, bl	971.4	100.00	1.46	6, bl	901.6	100.00	1.99	10, bl
Maximum	[6] 1,059.4				[6] 975.8				[6] 1,002.3			
C. U		[7] 42.38				[7] 39.03				[7] 40.09		
pH			[8] 7.29				[8] 7.13				[8] 7.37	

See footnotes at end of table.

TABLE 2.—*Effects of different nitrogen compounds (665 mg of N per liter) in an optimum solution* [1] *(dibasic) on the growth of Aspergillus niger at 35° C. for 4 days, when optimum heavy-metal concentrations were employed*—Continued

Element omitted	$NaNO_3$				$NaNO_3$ [2]			
	Yield per 2.5 g sucrose	Proportion of maximum yield obtained	Acidity at harvest	Sporulation	Yield per 2.5 g sucrose	Proportion of maximum yield obtained	Acidity at harvest	Sporulation
	Milligrams	*Percent*	*pH*		*Milligrams*	*Percent*	*pH*	
Fe	199.8	20.67	3.25	8, bl	240.3	25.05	3.36	6, bl
Zn	203.4	21.05	3.16	8, bl	280.6	29.25	2.97	6, bl
Cu	861.4	89.13	3.86	6, t	963.2	100.39	4.17	6, t
Mn	889.5	92.03	2.11	6, bl	824.6	85.93	2.05	0
Mo	104.7	10.84	3.30	6, bl	159.8	16.76	3.27	8, bl
None [5]	996.5	100.00	2.81	10, bl	959.5	100.00	3.19	10, bl
Maximum	[6] 983.6				[6] 965.6			
C. U		[7] 39.44				[7] 38.62		
pH			[8] 7.45				[8] 4.85	

[1] See footnote 1, table 1.
[2] See footnote 2, table 1.
[3] See footnote 3, table 1.
[4] See footnote 4, table 1.
[5] Trace-element concentrations (mg per liter) were as follows: With NH_4NO_3: Fe, 0.24; Zn, 0.26; Cu, 0.04; Mn, 0.03, and Mo, 0.03. With NH_4Cl: Fe, 0.20; Zn, 0.20; Cu, 0.05; Mn, 0.02; and Mo, 0.02. With urea: Fe, 0.24; Zn, 0.26; Cu, 0.04; Mn, 0.03; and Mo, 0.04. With $NaNO_3$: Fe, 0.24; Zn, 0.30; Cu, 0.08; Mn, 0.05; and Mo, 0.06. With $NaNO_3$ (acid): Fe, 0.20; Zn, 0.22; Cu, 0.04; Mn, 0.02; and Mo, 0.02.
[6] See footnote 6, table 1.
[7] See footnote 7, table 1.
[8] See footnote 8, table 1.

TABLE 3.—*Effects of different nitrogen compounds (665 mg of N per liter), in an optimum solution* [1] *(dibasic) on the growth of Aspergillus niger at 35° C. for 4 days, when optimum heavy-metal concentration and a 40-percent excess of potassium phosphate and magnesium sulphate were employed.*

Element omitted	NH_4NO_3				NH_4Cl				Urea			
	Yield per 2.5 g sucrose	Proportion of maximum yield obtained [3]	Acidity at harvest	Sporulation [4]	Yield per 2.5 g sucrose	Proportion of maximum yield obtained	Acidity at harvest	Sporulation	Yield per 2.5 g sucrose	Proportion of maximum yield obtained	Acidity at harvest	Sporulation
	Milligrams	*Percent*	*pH*		*Milligrams*	*Percent*	*pH*		*Milligrams*	*Percent*	*pH*	
Fe	276.9	26.31	1.93	4, bl	363.7	35.36	1.79	6, bl	243.8	26.69	2.54	2, bl
Zn	136.0	12.83	2.38	5, bl	34.8	3.39	2.63	4, bl	36.5	4.00	2.84	2, bl
Cu	886.0	84.19	2.82	2, w	970.7	94.38	1.42	1, w	909.5	98.46	2.48	2, br
Mn	870.0	82.66	1.65	2, bl	795.0	77.30	1.41	2, bl	822.1	89.98	2.27	2, bl
Mo	680.6	64.67	1.83	2, bl	1,017.6	98.93	1.42	2, bl	1,008.1	110.34	2.49	8, bl
None [5]	1,052.4	100.00	2.15	8, bl	1,028.5	100.00	1.44	4, l	913.6	100.00	2.26	8, bl
Maximum	[6] 1,070.1				[6] 1,036.0				[6] 1,142.7			
C. U		[7] 42.80				[7] 41.44				[7] 45.71		
pH			[8] 7.36				[8] 7.23				[8] 7.68	

See footnotes at end of table.

TABLE 3.—*Effects of different nitrogen compounds (665 mg of N per liter), in an optimum solution*[1] *(dibasic) on the growth of Aspergillus niger at 35° C. for 4 days, when optimum heavy-metal concentration and a 40-percent excess of potassium phosphate and magnesium sulphate were employed*—Continued

Element omitted	$NaNO_3$				$NaNO_3$[2]			
	Yield per 2.5 g sucrose	Proportion of maximum yield obtained	Acidity at harvest	Sporulation	Yield per 2.5 g sucrose	Proportion of maximum yield obtained	Acidity at harvest	Sporulation
	Milligrams	*Percent*	*pH*		*Milligrams*	*Percent*	*pH*	
Fe	259.5	24.60	3.27	4, t	266.9	26.87	3.32	4, bl
Zn	60.6	5.95	3.08	4, bl	119.7	12.06	2.98	2, bl
Cu	825.5	83.72	4.12	2, t	929.0	93.54	4.60	3, t
Mn	993.6	97.58	2.27	3, bl	865.1	87.10	2.04	0
Mo	81.0	7.95	3.05	3, bl	118.9	11.97	3.00	4, bl
None[5]	1,018.3	100.00	2.94	10, bl	994.9	100.00	3.33	10, bl
Maximum	[6] 1,035.5				[6] 1,005.6			
C. U.		[7] 41.42				[7] 40.22		
pH			[8] 7.46				[8] 4.93	

[1] See footnote 1, table 1.
[2] See footnote 2, table 1.
[3] See footnote 3, table 1.
[4] See footnote 4, table 1.
[5] See footnote 5, table 2.
[6] See footnote 6, table 1.
[7] See footnote 7, table 1.
[8] See footnote 8, table 1.

The data of table 1 may be summarized by the statement that compared to the yield obtained with the dibasic optimum (NH_4NO_3) the maximum yields were low only with urea and sodium nitrate (pH 7.45). Deficiency tests with the trace elements in these solutions yielded poorer results on the whole than with ammonium nitrate, but only in the case of molybdenum deficiency in both the alkaline and acid sodium nitrate solutions did a significant difference appear. The effects of molybdenum deficiency in the latter solutions were a very marked diminution in yield and sporulation. The cultures were typical in appearance of those suffering from a marked nutrient deficiency of such elements as iron, zinc, sulphur, nitrogen, magnesium, or phosphorus (*14*).

In table 2, with optimum concentrations of trace elements, as well as in table 3, where trace elements were in optimum concentration and potassium phosphate and magnesium sulphate were in moderate excess, the maximum yields with all solutions showed good agreement. The effects of trace-element deficiencies were quite similar to those in the experiments of table 1. Examination of these tables reveals that the molybdenum deficiency tests with urea and ammonium salt gave the poorest results, those with ammonium and nitrate (NH_4NO_3) gave results of intermediate value, and those with nitrate gave the most striking results. Neither moderate increase in acidity and trace elements nor increased quantities of the other essential elements modified these results appreciably.

Though for reasons that will be stated later it seemed quite certain that the difference in results with ammonium nitrogen and nitrate nitrogen were associated with a higher molybdenum requirement in nitrate solutions, these experiments were extended to include other salts containing ammonium or nitrate nitrogen. Tests were also made of the effects at a marked acidity, and of nitrogen supplied as asparagine. These results have been tabulated in tables 4 and 5.

TABLE 4.—*Miscellaneous experiments on the effects of different nitrogen compound- (665 mg of N per liter) in an optimum solution* [1] *(dibasic) on the growth of Aspergillus niger at 35° C. for 4 days*

Element omitted	$NaNO_3$ [2] (high acidity)				$Ca(NO_3)_2.4H_2O$				KNO_3			
	Yield per 2.5 g sucrose	Proportion of maximum yield obtained [3]	Acidity at harvest	Sporulation [4]	Yield per 2.5 g sucrose	Proportion of maximum yield obtained	Acidity at harvest	Sporulation	Yield per 2.5 g sucrose	Proportion of maximum yield obtained	Acidity at harvest	Sporulation
	Milligrams	*Percent*	*pH*		*Milligrams*	*Percent*	*pH*		*Milligrams*	*Percent*	*pH*	
Fe	319.9	34.98	3.12	6, bl	139.6	14.03	3.12	4, bl	235.5	22.88	3.26	6, bl
Zn	93.1	10.18	3.16	6, bl	121.0	12.17	2.99	4, bl	100.9	9.80	3.04	4, bl
Cu	947.1	103.56	3.23	4, t	879.0	88.40	3.28	2, y	1,047.7	101.77	3.87	8, t
Mn	526.7	57.59	2.07	0	539.2	54.22	3.14	0	859.3	85.48	2.11	0
Mo	245.7	26.86	3.02	6, bl	880.9	88.59	3.34	8, br	150.5	14.62	3.05	8, br
None [5]	914.5	100.00	3.05	9, bl	994.4	100.00	3.57	10, br	1,029.4	100.00	3.08	10, br
Maximum	[6] 1,067.4				[6] 1,021.8				[6] 1,069.8			
C. U		[7] 42.70				[7] 40.87				[7] 42.79		
pH			[8] 2.48				[8] 4.63				[8] 4.93	

Element omitted	$(NH_4)_2SO_4$				$Ca(NO_3)_2.4H_2O$			
	Yield per 2.5 g sucrose	Proportion of maximum yield obtained	Acidity at harvest	Sporulation	Yield per 2.5 g sucrose	Proportion of maximum yield obtained	Acidity at harvest	Sporulation
	Milligrams	*Percent*	*pH*		*Milligrams*	*Percent*	*pH*	
Fe	254.8	24.92	2.12	4, bl	66.4	6.59	3.06	2, bl
Zn	71.9	7.03	2.56	4, bl	99.0	9.82	2.98	2, bl
Cu	926.0	90.55	1.54	0	914.7	90.87	2.36	6, t
Mn	629.0	61.21	1.54	0	797.5	79.23	1.66	0
Mo	1,018.6	99.61	1.51	1, bl	938.0	93.19	2.72	8, bl
None [5]	1,022.7	100.00	1.52	2, bl	1,006.6	100.00	2.73	8, bl
Maximum	[6] 1,046.3				[6] 1,207.8			
C. U		[7] 41.85				[7] 48.31		
pH			[8] 5.08				[8] 4.47	

[1] See footnote 1, table 1.
[2] See footnote 2, table 1.
[3] See footnote 3, table 1.
[4] See footnote 4, table 1.
[5] See footnote 5, table 1.
[6] See footnote 6, table 1.
[7] See footnote 7, table 1.
[8] See footnote 8, table 1.

TABLE 5.—*Additional miscellaneous experiments on the effects of different nitrogen compounds (665 mg of N per liter) in an optimum solution* [1] *(dibasic) on the growth of Aspergillus niger at 35° C. for 4 days*

Element omitted	NH_4Cl+NaCl (0.2 percent)				Asparagine				$LiNO_3$			
	Yield per 2.5 g sucrose	Proportion of maximum yield obtained [2]	Acidity at harvest	Sporulation [3]	Yield per 2.5 g sucrose	Proportion of maximum yield obtained	Acidity at harvest	Sporulation	Yield per 2.5 g sucrose	Proportion of maximum yield obtained	Acidity at harvest	Sporulation
	Milligrams	*Percent*	*pH*		*Milligrams*	*Percent*	*pH*		*Milligrams*	*Percent*	*pH*	
Fe	386.0	38.73	1.73	5, bl	260.2	29.97	2.57	10, bl	170.3	22.62	3.33	4, bl
Zn	34.0	3.41	2.68	4, bl	151.6	17.47	2.61	10, bl	96.8	12.86	3.10	4, bl
Cu	936.0	93.94	1.42	2, w	1,110.9	127.96	2.52	4, t	803.6	106.73	3.93	2, w
Mn	730.8	73.34	1.39	2, bl	1,088.3	125.35	2.23	4, bl	784.9	104.25	2.09	0
Mo	979.5	98.30	1.40	3, bl	940.9	108.38	2.40	10, bl	110.8	14.71	3.09	6, bl
None [4]	996.5	100.00	1.40	6, bl	868.2	100.00	2.18	10, bl	752.9	100.00	3.62	8, bl
Maximum	[5] 1,051.5				[5] 1,136.6				[5] 997.6			
C. U		[6] 42.06				[6] 45.46				[6] 39.90		
pH			[7] 7.02				[7] 6.56				[7] 4.78	

Element omitted	$Mg(NO_3)_2.6H_2O$				NH_4NO_3 (purified with $CaCO_3$)			
	Yield per 2.5 g sucrose	Proportion of maximum yield obtained	Acidity at harvest	Sporulation	Yield per 2.5 g sucrose	Proportion of maximum yield obtained	Acidity at harvest	Sporulation
	Milligrams	*Percent*	*pH*		*Milligrams*	*Percent*	*pH*	
Fe	275.4	29.18	3.02	6, bl	18.8	1.59	2.85	2, bl
Zn	157.2	16.66	2.91	6, bl	12.5	1.05	2.94	2, bl
Cu	1,017.3	107.78	2.74	6, t	917.1	77.61	2.51	2, w
Mn	1,046.7	110.90	2.08	2, bl	1,056.3	89.40	1.67	2, bl
Mo	152.4	16.15	2.64	4, bl	1,067.7	90.36	2.15	8, bl
None [4]	943.8	100.00	2.50	10, bl	1,181.6	100.00	2.49	10, bl
Maximum	[5] 1,051.9				[5] 1,215.7			
C. U		[6] 42.08				[6] 48.63		
pH			[7] 4.51				[7] 6.94	

[1] See footnote 1, table 1.
[2] See footnote 3, table 1.
[3] See footnote 4, table 1.
[4] See footnote 5, table 1.
[5] See footnote 6, table 1.
[6] See footnote 7, table 1.
[7] See footnote 8, table 1.

A study of all the tables reveals that, except in the case of calcium nitrate, the omission of molybdenum resulted in the greatest loss in yield and sporulation with nitrate nitrogen and the least loss with ammonium nitrogen and organic nitrogen (urea, asparagine). Neither high acidity nor the possible influence of the cation introduced with the nitrate nitrogen seems to have been a factor. The extent of growth with deficiency of the trace elements other than molybdenum varied to about the same degree as in previous experiments, though it seemed on the whole to be somewhat larger than with ammonium nitrate. Though the solution with magnesium nitrate gave results that were quite poor with iron, zinc, manganese, and copper deficiencies, the results with molybdenum deficiency were still excellent.

Comparison of the deficiency yields with sodium nitrate at the three different levels of acidity brings additional confirmation of previous statements by the writer (*15*) that acidity decreases the optima or

increases the availability of the trace elements iron, zinc, copper, and manganese. Molybdenum, it is now noted, behaves similarly; it is also noted that the beneficial influence of acidity is limited at the highest pH value in the case of manganese. The combined deleterious effect of excess acidity and manganese deficiency on yield and sporulation becomes evident at even moderate acidities.

DISCUSSION

A comparison of the maximum yields obtainable with ammonium nitrogen and nitrate nitrogen in these experiments would indicate full nutritive equivalence of these forms of nitrogen. The maximum individual yield with ammonium nitrogen was 1,051.8 mg and with nitrate nitrogen 1,078.9 mg, the difference amounting to only 2.58 percent of the smaller number. The data, moreover, afford an explanation of previous claims to the contrary. Experimental evidence appearing to support this latter view can be readily obtained if the precaution of adjusting each of the other components for maximum efficiency is overlooked. However, tables 1 to 3 present unquestionable evidence that it is experimentally unsound to omit a check on each of the other known components of a nutrient solution before ascribing observed effects wholly to variations in even the better known constituents. Many of the effects attributed in the literature to variation in composition or concentration of nutrient salts of nitrogen, potassium, etc., are almost certainly due partly to chance variations in the essential trace elements which the salts contain as impurities.

No verification could be obtained of the claims for special effects of acidity, iron, copper, or manganese on the utilization of nitrates. Their effects on this process are quite similar to those of other nutrients. The action of increased acidity, as already pointed out, is to make more available the trace elements present so that growth can proceed further. This decrease in the optimum concentration of the trace elements might readily be attributed to the direct action of increased acidity though primarily due to the elements themselves. The sharply contrasting results with molybdenum in ammonium and nitrate solutions are unique and not shared by the other heavy metals studied.

The results with molybdenum might conceivably be due either to the relative freedom of nitrates from this element or to a greater requirement in the presence of nitrates. Chemical determination of the molybdenum content of the reagents would be the logical and definite method of deciding this question, but lack of time and facilities rendered this procedure impracticable. Other evidence nevertheless affords sufficient aid to enable one to make a decision.

The assumption of a greater molybdenum content in the ammonium salts supplying nitrogen is not substantiated by the impurity content of the other trace elements in these salts, since when they were employed in deficiency tests the percentages of maximum yield were approximately the same. Neither do the solutions employing nitrate nitrogen all require the addition of greater quantities of molybdenum to bring about maximum growth and sporulation. Lastly, spectroscopic examination failed to reveal the presence of molybdenum in the ammonium nitrate used, though a trace was found in the sucrose (*16*).

Similar results, too, were obtained with a sample of ammonium sulphate of spectroscopic purity. All the evidence available supports the assumption that nitrates act through their presence and not because of their relative freedom from molybdenum impurity; that is to say, the need of the fungus for molybdenum is greater when nitrogen is supplied as nitrate. Or, phrased differently, the trace of molybdenum unavoidably present in the nutrient solution suffices for a greater production of mass with ammonium nitrogen than with nitrate nitrogen.

It should not be assumed, however, that it is solely under such conditions that molybdenum is required. The omission of molybdenum from the nutrient solution causes a slight but definite diminution in yield with other sources of nitrogen than nitrate. With improved purity of reagents a still greater diminution in yield will probably be obtained with ammonium salts also when molybdenum is withheld. Though these results lead naturally to the assumption that molybdenum functions in the reduction processes of the fungus whereby nitrate is reduced to ammonia, molybdenum doubtless functions in other metabolic processes as well. If this viewpoint be correct, the possibility further suggests itself that the macroscopic appearance of the fungus when molybdenum is withheld may be due primarily to nitrogen starvation even though nitrate is present. The symptoms of molybdenum deficiency with nitrate nitrogen are quite similar to those of nitrogen deficiency, but this is true also of all the others except potassium, manganese, and perhaps copper. The symptoms of molybdenum deficiency may be found, therefore, to be somewhat different with ammonium nitrogen.

Interpolation here of some observations on the response of the fungus to molybdenum deficiency when nitrogen is supplied as calcium nitrate will not be amiss in view of the very frequent use of this salt in nutrient solutions for green plants. Both of the samples of this salt that were tested would appear to be contaminated with molybdenum to such an extent as to render them unfit for deficiency studies on this element. This condition is believed not to be accidental but to be due to the comparative difficulty of obtaining this salt in a sufficient degree of purity.[3] Lithium, sodium, potassium, magnesium, and even ammonium nitrate, of reagent grade, appear much more suitable for studies of molybdenum deficiencies. This variation in percentage of molybdenum impurity of different samples of nitrates may be a partial explanation, moreover, of their reported varying effectiveness in nutrient solution and as fertilizer.

The importance of the impurities present, even in the purest reagents now commercially available for use in studying the responses of plants to nutrient variations in culture, is greatly underestimated by many investigators. The many elements that may thus be accidentally included are listed in the tabulation that follows. Even this tabulation is inadequate, however, since the spectroscope is unsatisfactory foidentification of some of the chemical elements at concentrations bior logically significant. Tentatively, it is estimated that if an element is present in quantities greater than 1 part per billion one cannot be

[3] This opinion is fully substantiated by data obtained shortly after this manuscript was accepted for publication. Extreme differences between the molybdenum content of reagent calcium nitrate of different manufacturers is indicated; some samples contain traces of molybdenum ample for the needs of the organism, whereas others do not. Data not yet published show that marked decreases in yield occurred in certain instances with calcium nitrate and also with strontium nitrate and with barium nitrate when molybdenum in the nutrient solution was deficient in quantity.

reasonably certain that it is unessential. This degree of purity is seldom attainable at present. The only safeguards available are a thorough check of all components of the specific culture solution used and their employment in the minimum quantities sufficing for maximum growth under the conditions employed. Neglect of these precautions is undoubtedly the cause of many of the conflicting findings reported in the literature.

Impurities identified spectroscopically as present in some of the reagents[1] used in nutrient solutions for Aspergillus niger[2]

Compound:	*Contaminants identified spectroscopically*[3]
NH_4NO_3	Na, Mg, Ca, K(?).
K_2HPO_4	Al*, Pb*, Na*, Ca*, Mg, Ag.
$MgSO_4.7H_2O$	Na, Cu.
$FeSO_4.7H_2O$	
$ZnSO_4.7H_2O$	Fe, B(?), As, Mg*, Sn(?), Cu, Si, Na, Mn.
$CuSO_4.5H_2O$	Fe, Mn, Si, Mg, Ca, Pb.
$MnSO_4.2H_2O$	Na, Fe, Cu, Al, V, Cr, Si*, Mg*, Ca*.
Na_2MoO_4	Cu, Mn, Fe, Al, Ni, Mg, Ca*, K, Na, Mn, Si, Li, V(?).
Dextrose[4]	Li, Na, Sr, Ca, Rb, K, Mn, Al, Fe, Rh, Ni, Ag, Cu, Mg, Sn, B, Si.
$SrCl_2.6H_2O$	Na, Mg, Ba. Ru, Ti(?), Pt, Ir, Ca*, Mn, Pd, Si, Tl(?).

[1] These data were obtained by B. C. Brunstetter, associate biochemist, Division of Fruit and Vegetable Crops and Diseases, Bureau of Plant Industry, with a Bausch & Lomb large-size quarts spectrograph and carbon arc. No attempt was made to achieve prior chemical concentration or separation of the impurities, however.

[2] The following elements were found in the ash of *Aspergillus niger* felts grown in a dibasic optimum solution with the above chemicals: K, Mg, Fe, Cu, Mn, Na, Ca, Sr, Ba, Al, Pb, Ag. Tl(?), V(?).

[3] Strong traces are indicated by asterisks (*), doubtful by question marks.

[4] This compound was not employed but is inserted for the purpose of giving an idea of the probable impurities in sucrose. The data are quoted from Lockwood (*8*).

The relation ascertained to exist between nitrates and molybdenum in the metabolism of *Aspergillus niger* also possibly affords a definite chemical basis for explaining certain observations on green plants. Dittrich (*3*) found that extracts of beet tissue gave a negative test for nitrate reductase when the plants were supplied with ammonium nitrogen. This observation has been verified by Tiedjens and Blake (*17*) with extracts of apple tree roots. There would seem, therefore, to be a definite correlation in plants between utilization of nitrate nitrogen, presence of reductase, and high molybdenum requirement as contrasted with utilization of ammonium nitrogen, absence of reductase, and minimum need for molybdenum. It should be remembered in this connection that enzymatic deficiency in consequence of the absence of a suitable substrate has frequently been reported.

The direct determination of the relationship between reductase and molybdenum would be of considerable interest. This element may of course function purely as an inorganic catalyst after the manner reported by Kharasch et al. (*6*) for the oxidation of thioglycolic acid. On the other hand, molybdenum may serve to activate nitrate reductase, because it forms an integral part of the reductase molecule. The activation of urease by certain metal ions in the hydrolytic splitting off of ammonia from arginine is considered necessary for this reason by Kellerman and Perkins (*5*). In both the investigations just cited the metallic ions are required in traces of fractional parts per million only, and are therefore comparable as respects both type of reaction and concentration of metal ions to the requirements of plant metabolism. Moreover, even in vitro, specificity of effect was found

to exist in these reactions. That specificity was not found more complete in vitro is probably due to the limitation of a single reaction, whereas the organism may, and probably does, employ each essential element in several or more reactions simultaneously. Nor, on the other hand, is specificity absolute in the organism, as the phenomenon of nutrient substitution demonstrates.

CONCLUSIONS

Deficiency tests with *Aspergillus niger* in culture solutions indicate that ammonium nitrogen, nitrate nitrogen, and organic nitrogen (urea, asparagine) are equivalent in value for the nutrition of this fungus. It is necessary, however, to adjust correctly the concentration of the other essential components of the nutrient solution, particularly that of iron, zinc, copper, manganese, and molybdenum. Acidity is of minor importance and is effective chiefly through its action in aiding to minimize deficiencies of these trace elements. The response of the organism to molybdenum is unique in that it is definitely associated with the type of nitrogen nutrition. Molybdenum is required to a greater degree by the organism when nitrate is the source of nitrogen than when ammonium or organic nitrogen is the source. Though marked variations in molybdenum content of different lots were found to exist, striking deficiency results were obtained with many of the alkali and alkaline earth nitrates. It is considered, therefore, on the basis of these and of other facts reported in the literature, that molybdenum is essential for activation of nitrate reductase in the reduction processes whereby nitrates are reduced to ammonium for synthesis of amino acid and protein by the plant. It is suggested also that biological specificity is a result of chemical specificity of an element and presumably becomes more complete with increase in the number of reactions in which it simultaneously participates in the metabolism of the organism.

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THE TRANSLOCATION OF DERRIS CONSTITUENTS IN BEAN PLANTS[1]

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INTRODUCTION

In certain field tests with insecticides for the control of the Mexican bean beetle (*Epilachna varivestis* Muls.) the growth of the bean plants that formed after the application of a suspension of derris or cube in water appeared to receive some protection from infestation. It was suspected that this protection was due to the adsorption-absorption and translocation of the derris or cube constituents. With this in mind tests were undertaken to determine whether this phenomenon could be explained.

GENERAL METHODS OF PLANT TREATMENT

All the bean plants were grown under greenhouse conditions. At the beginning of all tests the pots were washed with water to prevent contamination, and in the last series fresh soil and new pots and stakes were used.

As soon as the first true leaves had formed and the buds of the first trifoliate leaves were forming, the plants were treated with the derris suspension, some by spraying with a compressed-air hand sprayer, some by painting the first true leaves with a camel's-hair brush, and some by painting only the stems. After the treatment the plants were staked and intermingled among untreated plants, with sufficient space between pots to prevent contamination. All moisture for plant growth was supplied by adding water to the pot saucer or at the base of the plant.

Pinto beans were used in certain larval feeding tests (A and B, table 1), and extracts from the same variety were used in tests with goldfish (A and B, table 2). Burpee Stringless Green-Pod beans were used in all the remaining tests.

FEEDING TESTS WITH MEXICAN BEAN BEETLE LARVAE

In all larval feeding tests but one the leaves were removed from the bean plants, and the feeding was conducted in a constant-temperature cabinet operated at 26.7° C. and 60-percent relative humidity, by a technique developed for toxicological studies of the Mexican bean beetle larvae.[3] The leaves were laid flat on a glass plate approximately 10 by 10 inches, with the lower surfaces upward and with the stems inserted in individual water reservoirs. An open glass feeding cell,

[1] Received for publication Aug. 14, 1937; issued February 1938.
[2] The authors wish to acknowledge many helpful suggestions from N. F. Howard and W. H. White during the course of this investigation.
[3] FULTON, R. A. A TECHNIQUE FOR THE TOXICOLOGICAL STUDIES OF MEXICAN BEAN BEETLE LARVAE. (In preparation.)

approximately 1¼ inches in diameter by 1¼ to 1½ inches high, was placed on each leaflet. One second-instar Mexican bean beetle larva, reared under laboratory conditions, was placed in each cell, except in the second test (B, table 1) when two larvae were used. They were confined by means of a screen-wire top. The plate was then placed in the constant-temperature cabinet.

In one test (A, table 1) the feeding was done on the plant under greenhouse conditions. The cells were placed on the leaves with the under surfaces upward, each leaflet resting on a small wooden block supported by a wire stirrup. One larva was placed in each cell and confined as described above. Third- and fourth-instar larvae collected from the field were used, but the larvae were in only fair condition, as it was late in the fall when the test was conducted.

In all tests the larvae were allowed to feed for a definite time, at the end of which examinations were made and mortality and area of leaf surface consumed were recorded. The leaf surface removed by each larva was determined by placing a "flexible glass" guide marked in 2-mm squares directly over the leaf. The larvae were transferred to new leaves when necessary, and to prevent contamination during the transfer, hands were washed with soap and water and clean camel's-hair brushes were used for each type of treatment.

The results of these tests are summarized in table 1.

TABLE 1.—*Feeding of Mexican bean beetle larvae on new growth of bean plants previously treated with a suspension of derris in water* [1]

Test	Type of treatment	Trifoliate leaves used	Time after treatment when feeding tests were begun	Larvae used	Duration of feeding tests	Average total leaf area consumed per larva	Reduction of feeding area compared with that of untreated plants	Mortality
			Days	*Number*	*Hours*	*Sq. millimeters*	*Percent*	*Percent*
A	Plants sprayed	First	12	16	74	421	51	0
	Untreated plants	do		[2] 4	74	866		0
B	Plants sprayed	do	12	30	72	143	24	6.6
	Leaves painted	do	12	24	72	115	39	8.3
	Stems painted	do	12	24	72	125	34	4.2
	Untreated plants	do		24	72	188		4.2
C	Leaves painted	do	11	15	144	687	37	0
	Stems painted	do	11	15	144	761	30	6.6
	Untreated plants	do		15	144	1,092		0
D	Leaves painted	Second	18	15	96	194	42	0
	Stems painted	do	18	15	96	220	34	0
	Untreated plants	do		15	96	335		0
E	Leaves painted	Third	27	15	96	267	32	6.6
	Stems painted	do	27	15	96	278	29	0
	Untreated plants	do		15	96	393		0

[1] In test A the rotenone content of the derris suspension was 0.025 percent; in all other tests the rotenone content of the suspension was 0.05 percent.
[2] 1 larva escaped during the first 24 hours.

The data show that the average leaf area removed per larva on new growth of treated plants is definitely reduced, as compared with the feeding area removed from untreated plants. Reductions in feeding area, 51 and 24 percent, respectively, occurred when first trifoliate leaves from sprayed plants were used (tests A and B). There were reductions of 39 and 37 percent, respectively, in the feeding on first trifoliate leaves from leaf-painted plants (tests B and C). When new

growth from stem-painted plants was used, the average reductions in leaf area consumed were 34 and 30 percent, respectively (tests B and C). When second trifoliate leaves from the same plants used in the preceding tests were fed (test D), reductions of 42 percent on leaf-painted and 34 percent on stem-painted plants occurred. Third trifoliate leaves from the same group of plants used in the two preceding tests were fed (test E), and even though the original treated area was much less in proportion to the total area of new growth after treatment, reductions of 32 and 29 percent occurred on leaf-painted and stem-painted plants, respectively.

When the daily totals were compared, it was noted that the area consumed per larva from leaf-painted plants was consistently higher during the first 24 hours. In the case of the stem-painted plants the area consumed on the first day was smaller than on the days of subsequent observations (after 2 to 4 days). At the close of the tests the total area consumed was greater on stem-painted plants than on leaf-painted plants.

TOXICITY TESTS WITH GOLDFISH

For the biological tests with goldfish (*Carassius auratus*) extracts were prepared from leaves of the same plants that were used in the feeding tests. This was done by extracting the leaf material with chloroform to eliminate excessive chlorophyll in the final preparation. The chloroform extract was evaporated to dryness on a steam bath and the residue steeped with an excess of acetone. The acetone solution was then evaporated and a known quantity of acetone added to this residue.

The tests with goldfish were made in a manner similar to that described by Gersdorff.[4] Aliquots of the acetone solution were added to known quantities of water at the rate of 1 cc per liter. In the first test recorded (A, table 2) 1,300 cc of water was used, and the solution was divided between two jars, 650 cc in each. Three fishes were placed in each jar. In the remaining tests 1,200 cc of water was used, divided between two jars, 600 cc in each, and two fishes were placed in each jar. The jars were then placed in a constant-temperature cabinet operating at 26.7° C. Observations were made at 30-minute intervals and the time of death was recorded. The results are shown in table 2.

In the first test (A) leaves were removed from the new growth of plants previously painted with a suspension of derris in water. The leaves were dried and an extract was prepared from 350 g of the material. This extract killed all the fishes in an average time of 220 minutes. The fishes in the solution prepared from dried leaves of the new growth of untreated plants were all alive and appeared normal at the end of 1,320 minutes.

Extracts prepared from first trifoliate leaves of leaf-painted and stem-painted plants (test B) killed all the fishes used in average times of 135 and 165 minutes, respectively. All the fishes were alive after 1,620 minutes in the solution prepared from untreated plants.

A third test (C) was performed to determine whether toxic material could be detected in the first trifoliate leaves by crushing the leaves and macerating them with water. The water extract was drained,

[4] GERSDORFF, W. A. A METHOD FOR THE STUDY OF TOXICITY USING GOLDFISH. Jour. Amer. Chem. Soc. 52: 3440-3445, illus. 1930.

filtered through cotton, and then made up to the same volume as used in other tests. All fishes were dead in the water extract of leaves from leaf-painted plants in an average time of 445 minutes and in the water extract of stem-painted plants in 482 minutes. All fishes in the water extract from leaves of untreated plants appeared normal at the end of 1,340 minutes.

TABLE 2.—*Toxicity to goldfish of extracts of new growth of bean plants previously treated with a suspension of derris in water* [1]

Test	Type of treatment	Trifoliate leaves used for extracts	Weight of leaves	Time after treatment when toxicity tests were begun	Goldfish used	Average weight of goldfish	Average time before death	Mortality
			Grams	*Days*	*Number*	*Grams*	*Minutes*	*Percent*
A [2]	Leaves painted	First	350	26	6	3.7	220	100
	Untreated plants	do	257		6	2.3		0
B	Leaves painted	do	87.5	11	4	1.91	135	100
	Stems painted	do	97.6	11	4	2.77	165	100
	Untreated plants	do	93.5		4	2.12		0
C [3]	Leaves painted	do	94	11	4	2.46	445	100
	Stems painted	do	89	11	4	2.18	482	100
	Untreated plants	do	65		4	2.56		0
D	Leaves painted	Second	114	18	4	1.78	105	100
	Stems painted	do	116	18	4	1.64	202	100
	Untreated plants	do	123		4	1.18		0
E	Leaves painted	Third	87	27	4	1.41	112	100
	Stems painted	do	92	27	4	1.86	462	100
	Untreated plants	do	85		4	1.45		0

[1] Derris suspension of 0.05 percent rotenone content was used in all tests except A, where a suspension of 0.25 percent rotenone was used.
[2] Leaves were weighed dry; in all other tests they were weighed green. In this test all the treated leaves had dropped from the plants when untreated leaves were picked.
[3] Solutions prepared by crushing the leaves in distilled water.

Extracts prepared from second trifoliate leaves (test D) of the same plants used in the two preceding tests killed all the fishes in the leaf-painted and stem-painted solutions in 105 and 202 minutes, respectively. At the end of 420 minutes all fishes in the untreated plant extract appeared normal.

In the final test with goldfish (E) third trifoliate leaves were removed from the same plants that were used in the three preceding tests. All the fishes were dead in an average time of 112 minutes in the extract from leaf-painted plants, whereas in the extract from stem-painted plants all were not dead until after an average of 462 minutes. All fishes in the untreated plant extract appeared normal at the end of 1,130 minutes.

The results recorded above show that toxic ingredients are present in the new growth of plants previously treated with suspensions of derris in water.

ISOLATION AND IDENTIFICATION OF ROTENONE

In one case rotenone was actually isolated from the plant material. Eight hundred grams of dry leaves from the new growth that formed after the application of a suspension of derris in water (0.025 percent of rotenone) was extracted with chloroform. The dried leaf material was covered with chloroform and allowed to stand at room temperature in subdued light for 3 days. At the end of that time the chloroform

solution was removed and the leaf material was again covered with chloroform. By using a known weight of leaf material, it had previously been determined that the cold method of extraction reduced the chlorophyll content of the extract. The chloroform extracts were evaporated to dryness and the residue was removed with carbon tetrachloride. The carbon tetrachloride solution was allowed to stand for several days in an ice box maintained at approximately 4° C. At the end of that time small needlelike crystals appeared in the solution. These crystals were washed with a small quantity of cold carbon tetrachloride, recrystallized from acetone to convert any solvate present to rotenone, and the melting point was then determined. Only 8 mg of this material was recovered from 800 g of dried new-growth leaves. The crystals gave the characteristic test with the Gross-Smith [5] colorimetric method, and had a melting point of 163.5° C., or approximately the melting point of rotenone (163° C.). The crystals were also tested by the use of the blue color test of Durham and they gave a color similar to that produced by bromothymol blue indicator at pH 7.2.

One milligram of this material was added to 650 cc of water containing 0.6 cc of acetone as a dispersing agent and tested with three goldfish at 26.7° C. All the fishes were killed in an average time of 77 minutes.

SUMMARY AND CONCLUSION

A definite retarding in the feeding of Mexican bean beetle larvae has been observed on the new growth of bean plants that have been treated with derris powder. First, second, and third trifoliate leaves, formed after the first pair of true leaves had been treated, were found to be less palatable to bean beetle larvae than similar leaves from untreated plants.

Extracts prepared from first, second, and third trifoliate leaves formed after the application of derris powder to the first pair of true leaves caused 100-percent mortality to goldfish. Extracts prepared from first, second, and third trifoliate leaves from untreated plants were not toxic.

A water extract prepared by macerating new growth of bean plants that had been treated with derris was fatal to goldfish.

In one case, where a large quantity of new-growth material was available, a crystalline substance resembling rotenone was isolated. The material when purified had a melting point of 163.5° C., gave the characteristic color with the Gross-Smith method, and the blue color test of Durham. One milligram of this substance in 650 cc of water at 26.7° C. killed three goldfish in an average of 77 minutes.

These experiments demonstrate that derris constituents are translocated from the outer surfaces of leaves to first, second, and third trifoliate leaves formed after the application of derris powder in water suspension to the first true leaves and stems of bean plants.

[5] GROSS, C. R., and SMITH, C. M. COLORIMETRIC METHOD FOR THE DETERMINATION OF ROTENONE. Jour. Assoc. Off. Agr. Chem. 17: 336–339. 1934.

QUANTITATIVE INJECTION AND EFFECTS OF NICOTINE IN INSECTS[1]

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INTRODUCTION

Nicotine is used as an insecticide chiefly for the control of small, soft-bodied, sucking insects. As ordinarily applied in sprays or dusts, it is ineffective against many species of caterpillars, beetles, flies, etc., either because it does not reach the vital parts of these insects or because it is not sufficiently toxic to them. It is therefore of interest to know whether certain species that are considered resistant to nicotine are actually susceptible when this material is introduced by injection into the body cavity. This study dealt with the improvement of apparatus for the quantitative injection of solutions into insects, the comparative susceptibility of various species to nicotine, the effect of age of insects on susceptibility, the difference between the toxicity of nicotine and that of nicotine sulphate, and other toxicological problems.

INJECTION APPARATUS, MATERIAL, AND METHODS

An attempt was made to use apparatus more accurate and more easily handled than any yet designed to feed or to inject quantitative doses into insects. A micrometer syringe (fig. 1, *A*), described by Trevan (*7*) [2], was used apparently for the first time to inject measured volumes of an insecticide into insects, although Storey (*6*) had previously used it to inject plant viruses into leafhoppers. This apparatus consists simply of an ordinary hypodermic syringe (*s*), the plunger of which is actuated by the head (*h*) of the micrometer (*m*), which is fastened to the syringe by a clamp (*c*). It was calibrated with distilled water in the Volumetric Section of the Bureau of Standards. A movement of the micrometer head through five divisions delivered 0.00124 cc (1.24 mm^3) with an error of less than 4 percent. A 27-gage steel hypodermic needle (*n*) 30 mm long was used on the syringe.

Various means of mounting and manipulating the syringe were tried. The arrangement illustrated in figure 2, *A*, was adopted, the syringe (*s*) being rigidly clamped in a horizontal position on the pillar (*mp*) of a dissecting microscope. The syringe was moved up or down by rack and pinion and in a horizontal plane by pushing the microscope base (*mb*) with both hands. Under the micrometer head (*h*) a wooden support (*ws*) was mounted to steady the hand while turning the micrometer screw.

[1] Received for publication August 4, 1937; issued February 1938.

[2] Reference is made by number (italic) to Literature Cited, p. 921.

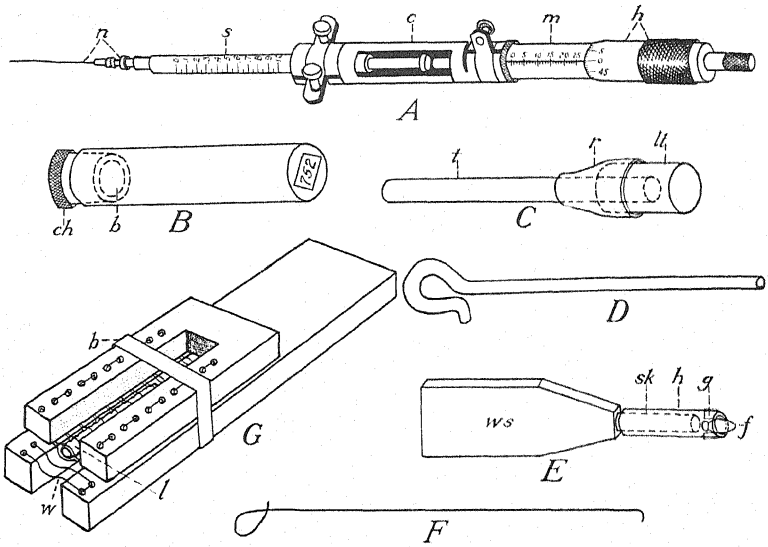


FIGURE 1.—Micrometer syringe and other devices used to inject insects: *A*, Micrometer syringe showing hypodermic needle (*n*), syringe (*s*), clamp (*c*), micrometer (*m*), and micrometer head (*h*); *B*, shell vial closed with a hollow cork stopper (*b*) whose outer end is covered with cheesecloth (*ch*); *C*, device to remove flies from vials, consisting of small glass tube (*t*), larger glass tube (*lt*), and rubber tube (*r*); *D*, steel rod to transfer flies; *E*, device to hold fly (*f*) during injection, consisting of wooden support (*ws*), its shank (*sk*), glass holder (*h*), and gummed cloth (*q*) to constrict opening in holder; *F*, wire to remove fly from glass holder; *G*, device to hold larva (*l*) during injection, showing fine wire grids (*w*) and rubber band (*b*) to hold the frames together.

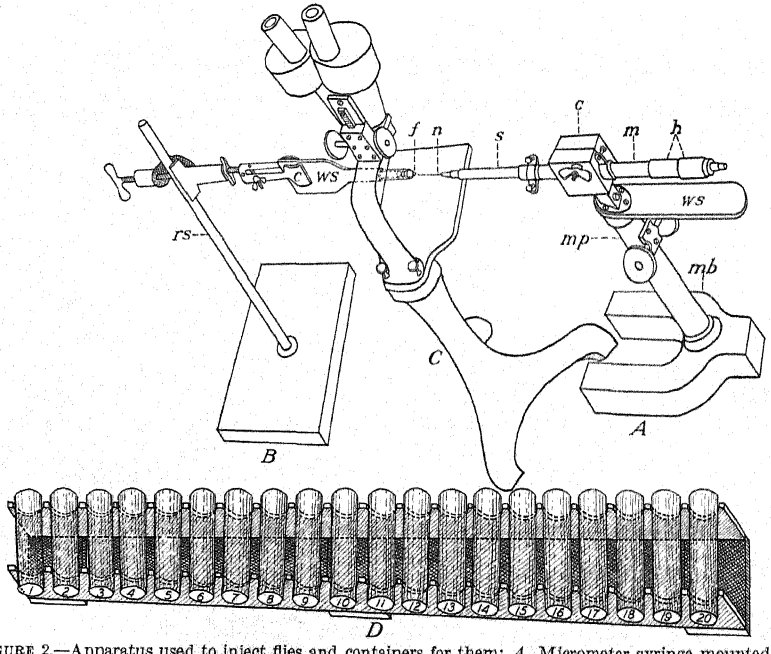


FIGURE 2.—Apparatus used to inject flies and containers for them: *A*, Micrometer syringe mounted on pillar (*mp*) of dissecting microscope, showing base (*mb*) of microscope, wooden support (*ws*), needle (*n*), syringe (*s*), wooden clamp (*c*) to hold syringe, micrometer (*m*), and micrometer head (*h*); *B*, ring stand (*rs*) and clamp (*c*) to hold wooden support (*ws*) and fly (*f*); *C*, binocular microscope; *D*, rack and shell vials in which the treated flies were kept.

Adults and larvae of the green-bottle blowfly (*Lucilia sericata* Meig.), the black blowfly (*Phormia regina* Meig.), and the blue-bottle blowfly (*Calliphora erythrocephala* Meig.) were used in most of the experiments. They were reared in the manner already described by the writer (*4*). Mature or nearly mature larvae of the following lepidopterous species were used in other tests: Eastern tent caterpillar (*Malacosoma americana* (F.)), codling moth (*Carpocapsa pomonella* L.), silkworm (*Bombyx mori* L.), and southern armyworm (*Prodenia eridania* (Cram.)). Nearly 4,000 insects were injected, and when large numbers of a species were used for a single solution the figures were treated statistically.

Solutions of pure nicotine and of nicotine sulphate in distilled water were prepared for the writer in the Division of Insecticide Investigations. These solutions were diluted with distilled water by the writer to make the following percentage concentrations of nicotine: 4.0, 3.0, 2.0, 1.5, 1.0, 0.8, 0.5, 0.25, 0.125, 0.062, and 0.031. A solution of each concentration was stored in a small bottle wrapped with black paper. Distilled water was used for check injections.

Shortly before a series of injections a sufficient number of ventilated, stoppered vials (fig. 1, *B*), 18 by 75 mm, were weighed collectively. The flies were then caught, one fly of known sex to a vial, by placing an unstoppered vial over the desired fly in the rearing cage. The stoppered vials were weighed again, and the average weight of a fly was calculated. A set of flies consisted of 20 individuals, usually 10 males and 10 females. The vials holding them were placed on a rack, shown in figure 2, *D*.

To inject a fly it was necessary to hold it mechanically under a binocular microscope (fig. 2, *C*). This was done as follows: The fly was removed from its vial by means of a glass tube (fig. 1, *C*). The larger tube (*lt*) was inserted into the vial, and the fly was made to pass through it into a smaller tube (*t*), from which it was forced by a steel rod (*D*) into a glass holder (*E*, *h*). The shank (*E*, *sk*) of a wooden holder (*ws*) was finally thrust full length into the holder, thus forcing the abdomen of the fly (*f*) through the constricted end to the exterior. Two sizes of holders were used, according to the size of the fly injected. Similar holders for queen bees have been described by Nolan (*5*, *p. 21*).

The flies were usually injected on the left side between the second and third abdominal segments halfway between the ventral and dorsal midlines. The needle was inserted the width of the second segment, allowed to remain for 15 seconds, and then pulled out slowly. The fly was immediately removed from the holder by a wire with a hooked end (fig. 1, *F*) and placed in its vial. About an hour was required to inject 20 flies. Each vial, containing an injected fly and sugar solution on cotton, was replaced on the rack for observation. During the period of injection the temperature of the vials was held at about 29° C., but thereafter it was uncontrolled and fluctuated with room temperature. Hockenyos and Lilly (*1*) determined that temperature was not an important factor when they injected nicotine into caterpillars.

During injection the larger larvae were held between two wooden frames with wire grids (fig. 1, *G*, *w*). The lower frame was extended beyond the grid to provide a means for clamping it to the ring stand. A larva was placed on the grid of the lower frame, and the grid of the

upper frame was placed over it. The two frames were held together with a rubber band (*G, b*) so placed that the larva was not injured by compression but was prevented from squirming loose. Smaller larvae were held between the grid of the upper frame and the extension of the lower frame. The needle of the syringe was inserted in the side of the larva between the fourth and fifth abdominal segments, counting from the posterior end. To prevent bleeding, the needle was left in the insect for 1 minute and then gently removed. The injected larvae were given food and confined for observation in small wire-screen cages.

Several kinds of records were taken. Longevity records, or counts of the number of dead insects, were made at 4 p. m., and three times daily thereafter, at 9 a. m., 12 m., and 4 p. m., until all the insects were dead. In some cases, for the sake of greater accuracy, counts were made hourly. The average length of life of the insects in the set was then calculated. At the same time activity records were taken on the following basis: No movement was represented by 0, very slight movement by 1, slight movement 2, considerable movement (able to stand on feet) 3, fair activity 5, nearly normal activity 7, and normal activity 10. These figures were then averaged on a percentage basis. Insects that were apparently lifeless were warmed under an electric lamp to bring out any latent activity. Finally, a paralysis record was kept to measure the duration of paralyzation; it included the first-reaction time, or the period after injection before the insect showed the first signs of life, and the revival time, or the period after injection before the fly was able to get upon its feet.

COMPARATIVE SUSCEPTIBILITY OF VARIOUS SPECIES OF INSECTS

A long series of tests was conducted to determine the comparative susceptibility, on the basis of body weight, of various species and the sexes to nicotine. Both nicotine and nicotine sulphate were injected, but the quantities are expressed as pure nicotine. An effort was made to keep the activity between 5 and 6 percent and the longevity, based on 100-percent mortality, at about 24 hours. The susceptibility was expressed as the body weight of the insect that was killed by 1 mg of pure nicotine.

The data for these tests are given in table 1. Since 2,727 mg of *Lucilia* males and 302 mg of *Phormia* larvae were killed by 1 mg of nicotine, the flies were about nine times as susceptible as the larvae. *Lucilia* males were considerably more susceptible than *Lucilia* females; *Calliphora* males and females were practically the same, but much less susceptible than *Lucilia*; codling moth larvae were about equal in susceptibility to *Lucilia* females; and the other larvae were much less susceptible than any of these insects. It is difficult, and sometimes impossible, to kill the southern armyworm by applying nicotine compounds to foliage, and the figure given in the table bears this out. The susceptiblity of the larval forms, except that of the codling moth, was about half that of the imaginal forms.

Although 120 tent caterpillars were also used in these tests, the figures are not included in table 1. If the dosage given to them had been doubled, their susceptibility would probably have been similar to that of the silkworm.

TABLE 1.—*Comparative susceptibility of various species of insects and their sexes to nicotine, based on 100-percent mortality*

Species and sex	Insects	Average weight of insects	Nicotine injected per insect	Average longevity	Average activity	Body weight of insects killed by 1 mg of nicotine
	Number	*Mg*	*Mg*	*Hours*	*Percent*	*Mg*
Lucilia, males	100	27	0.0099	23.7	5.4	2,727
Lucilia, females	90	38	.0248	26.4	6.1	1,532
	90	42	.0372	24.5	5.2	1,129
Calliphora, males	100	69	.0744	21.6	5.0	927
Calliphora, females	100	77	.0843	26.1	5.3	913
Codling moth larvae	60	44	.0372	20.3	6.1	1,182
Silkworms (fourth-instar)	60	517	.7440	27.6	6.1	695
Southern armyworms	20	625	1.2400	40.3	6.7	504
Phormia larvae (full-grown)	40	75	.2480	21.3	5.7	302

Numerous control tests, in which distilled water was used, were likewise conducted. Doses of it equal to those of the insecticides had little or no effect on the insects. The longevity was practically the same as that of untreated insects and the activity was 100 percent or nearly so.

EFFECT OF AGE ON SUSCEPTIBILITY OF FLIES

Earlier tests indicated that the age of the injected flies affected their susceptibility to nicotine, and that if more consistent results were to be obtained greater attention would have to be given to the age of the insects treated. A series of tests was therefore conducted with 20 *Phormia* flies, 10 males and 10 females, of various ages. A dose of 1.24 mm^3 of 3-percent nicotine solution was first tried to see if curves could be drawn representing the daily toxicity, based on longevity and activity records, during the lifetime (24 days or more) of the flies. This concentration was too high, however, for the curves (fig. 3, *a* and *b*) quickly ascended from the first to the fourth day and then quickly descended to the sixth day. A dose of 1.24 mm^3 of a 2-percent solution was next tried. This concentration produced curves (*c* and *d*) which rose quickly to the fifth day, but descended less rapidly to the twelfth day, and thereafter it was too high, for the activity had reached 3.3 percent and the longevity had reached 17 hours, both of which figures were the lowest that could be recorded on the scale used. After the twelfth day 0.5- and 0.25-percent solutions were used, and the average longevity was 17 hours on the fourteenth and eighteenth days, respectively. Flies 14 days old were therefore killed with 0.0031 mg of nicotine (0.25-percent solution) in 17 hours, while flies 5 days old were killed with 0.0248 mg (2-percent solution) in 22.2 hours. The old flies were more than eight times as susceptible as the young ones. The control insects, injected with distilled water, were affected little or not at all, even after the twelfth day, while those injected with nicotine were quickly paralyzed and soon died.

The flies were most resistant on the fourth and fifth days, but thereafter their susceptibility increased with age. Upon emerging as adults the flies were weak, but they gradually gained strength and

on the fourth day began to lay eggs. This information may explain why the curves ascend from the first to the fifth day, but the increasing age of the flies certainly explains why the curves descend thereafter.

The comparative susceptibility of *Phormia* flies of different ages to various concentrations of nicotine was also studied from hourly longevity records. In this case the susceptibility ratio was obtained by dividing the body weight of the insects by the weight of nicotine

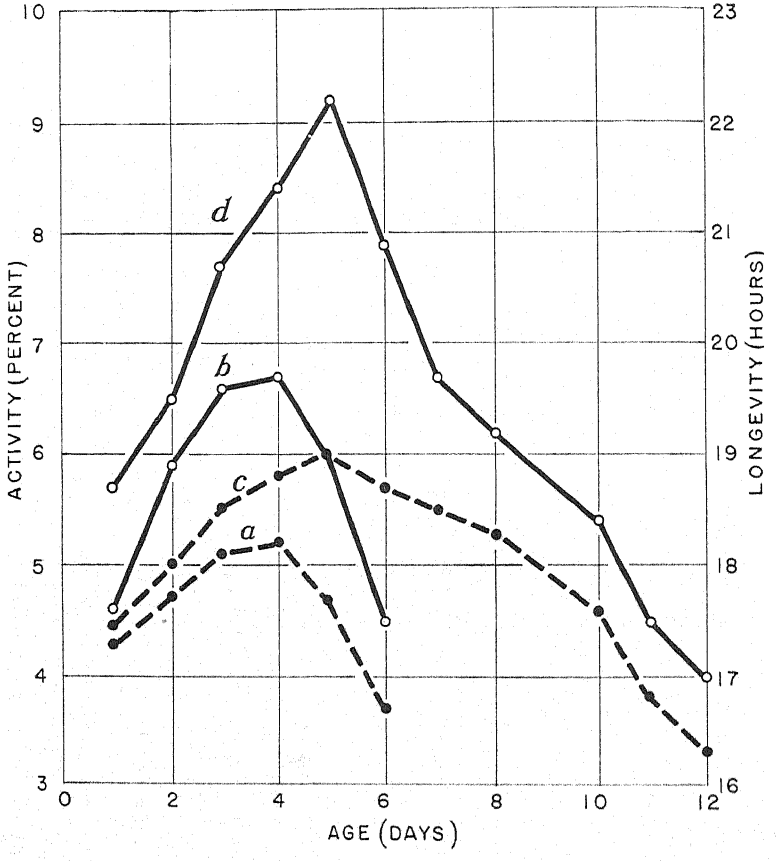


FIGURE 3.—Effect of age of flies (*Phormia*) on toxicity of nicotine: *a*, Activity, and *b*, longevity, with 3-percent solution; *c*, activity, and *d*, longevity, with 2-percent solution.

injected and then dividing this quotient by the longevity. The results are shown in table 2. Flies 5 days old are shown to be more resistant than flies 3 days old. Flies 5 days old showed no difference in toxic effects when injected with the same quantity of nicotine in 1- and 3-percent solutions. Both the longevity figures and the susceptibility ratios show that the males were more susceptible than the females. On the basis of milligrams of nicotine injected, the 16-day-old flies were six times as susceptible as the 3- and 5-day-old flies, whereas on the basis of the susceptibility ratios they were nearly seven times as susceptible.

TABLE 2.—*Comparative susceptibility of Phormia flies of different ages to various concentrations of nicotine, based on hourly longevity records*

Age of flies (days)	Weight of flies		Nicotine solution injected			Longevity of flies			Ratio of susceptibility	
	Females	Males	Concentration	Dose	Content of pure nicotine	Females	Males	Average	Females	Males
	Mg	*Mg*	*Percent*	*Mm³*	*Mg*	*Hours*	*Hours*	*Hours*		
3	42	38	3	1.24	0.0372	9.3	6.5	7.9	121	157
5	45	40	3	1.24	.0372	9.6	8.3	8.9	126	130
5	45	40	1	3.72	.0372	9.5	8.0	8.8	127	134
16	50	------	.5	1.24	.0062	9.5	------	------	849	------

COMPARISON OF TOXICITIES OF NICOTINE AND OF NICOTINE SULPHATE

Early tests with 2- and 3-percent solutions indicated that nicotine was more toxic to *Lucilia* than nicotine sulphate. On the basis of 100-percent mortality the activity records showed the free nicotine to be 14 percent more toxic than the nicotine salt, whereas the longevity records showed it to be only 8 percent more toxic. On the basis of 50-percent mortality the nicotine solution was 8.4 percent more toxic, according to the longevity records. From later activity records based on 100-percent mortality it was determined statistically that the nicotine solutions were not more toxic to female *Lucilia* than the nicotine sulphate solutions. The methods used allowed too many large deviations, and later it was ascertained that the nicotine solutions were the more toxic. In all instances the solutions of nicotine and nicotine sulphate were prepared on the basis of equal nicotine content.

In the hope of getting more accurate results, the toxicities of nicotine and nicotine sulphate were compared by the paralysis method. A dose of 1.24 mm^3 of 0.25-percent solution was injected into each fly of five sets of *Phormia* females and two sets of *Phormia* males. For the males the average first-reaction time for nicotine was 17.9 minutes and for nicotine sulphate 15.8 minutes; the average revival time for nicotine was 44.9 minutes and for nicotine sulphate 42.6 minutes. These figures show nicotine to be the more toxic.

The data for the females are given in table 3. According to the first-reaction time nicotine is 22.2 percent more toxic than nicotine sulphate, but according to the revival time it is 26.5 percent more toxic. These figures may be considered reliable, because, according to the statistical test, the probability of such differences occurring by chance is only 1 in 100.

The pH values of the solutions of nicotine and nicotine sulphate differed. Hockenyos and Lilly (*1*) determined that the effect of varying the acidity was very slight. In control tests the writer used potassium hydroxide and hydrochloric acid in distilled water and concluded that hydrogen-ion concentration alone had little effect. Levy's saline solution was slightly better than distilled water for control tests, but insecticides are never dissolved in it. Other tests in which large volumes of distilled water were injected into flies showed that the insects occasionally tolerated a dose equal to one-fourth the body weight.

TABLE 3.—*Difference between toxicities of nicotine and of nicotine sulphate to Phormia females as determined from paralysis records*

Group no.	First-reaction time		Revival time	
	Nicotine	Nicotine sulphate	Nicotine	Nicotine sulphate
	Minutes	*Minutes*	*Minutes*	*Minutes*
1	13.4	11.6	47.3	33.2
2	14.6	13.1	44.9	32.7
3	16.1	13.2	35.9	25.0
4	18.4	11.3	33.4	30.4
5	14.4	13.7	32.1	31.9
Mean [1]	15.5	12.6	38.7	30.7
Difference		2.8 ± 1.24		8.1 ± 2.59
Probability		.01		>.01
Total samples—number	46	50	43	46

[1] Mean of total number of samples in the five groups.

COMPARISON OF TOXICITIES OF NICOTINE IN DISTILLED WATER AND OF NICOTINE SULPHATE IN POTASSIUM HYDROXIDE SOLUTION

To free the nicotine in the 0.25-percent nicotine sulphate solution, a 0.1-percent solution of potassium hydroxide was used instead of distilled water to reduce the 20-percent solution of nicotine sulphate to this dilution. In controls a 0.1-percent solution of potassium hydroxide produced no signs of paralysis. In comparative tests five sets of *Phormia* males and females were injected with this solution and with a 0.25-percent solution of nicotine in distilled water. The results are given in table 4. The differences between the average results obtained were slight and not significant. The freed nicotine in the nicotine sulphate solution was, therefore, equally as toxic as the other free-nicotine solution.

TABLE 4.—*Difference between toxicities to Phormia males and females of nicotine in distilled water and of nicotine sulphate in potassium hydroxide solution as determined from paralysis records*

Group no.	First-reaction time		Revival time	
	Nicotine	Nicotine sulphate	Nicotine	Nicotine sulphate
	Minutes	*Minutes*	*Minutes*	*Minutes*
1	13.2	14.6	40.4	38.4
2	9.4	11.8	39.0	39.7
3	15.3	13.4	43.4	41.7
4	13.3	19.8	43.2	37.6
5	14.2	14.9	37.8	40.1
Mean [1]	13.1	14.9	40.8	39.5
Difference		1.8±1.06		1.3±2.96
Probability		>.20		>.60
Total samples—number	50	50	42	42

[1] Mean of total number of samples in the five groups.

A STUDY OF SYNERGISM

Levine and Richardson (2) made a study of synergism by injecting into cockroaches nicotine solutions made up in 0.1-molar solutions of

chlorides and bicarbonates of potassium and sodium and in distilled water (the control). Three of these salts increased the toxicity of nicotine, but potassium chloride was the best for this purpose. The writer compared the toxicity of nicotine solutions in distilled water and in 0.1-molar potassium chloride.

Three sets of *Calliphora* females, 13, 14, and 15 days old, were injected with 0.25-percent solutions, the dose being 2.48 mm^3, or 0.0062 mg of pure nicotine. The solutions were prepared by diluting one portion of the 20-percent nicotine solution with distilled water and another portion with 0.1-molar potassium chloride. The potassium chloride solution, used alone as a control, caused no sign of paralysis. As judged by the revival time, which is more reliable than the first-reaction time, the nicotine diluted with the potassium chloride solution was 10.4 percent more toxic than that diluted with distilled water, but when the figures, including many large deviations, were treated statistically there was no significant difference (table 5). If 100 flies, instead of 30, had been injected with each solution, the difference between the means might have been significant.

TABLE 5.—*Difference between toxicities to Calliphora flies of nicotine dissolved in distilled water and of nicotine dissolved in 0.1-molar potassium chloride solution*

Group no.	First-reaction time		Revival time	
	Nicotine in distilled water	Nicotine in potassium chloride solution	Nicotine in distilled water	Nicotine in potassium chloride solution
	Minutes	*Minutes*	*Minutes*	*Minutes*
1	11.3	10.3	37.1	43.3
2	10.6	12.0	39.3	40.8
3	11.2	9.5	39.1	43.4
Mean [1]	11.0	10.6	39.5	42.5
Difference		0.4±1.12		4.0±5.74
Probability		.60		.40
Total samples—number	30	30	28	28

[1] Mean of total number of samples in the 3 groups.

A STUDY OF MOTOR PARALYSIS

To ascertain more accurately the effects of nicotine on flies, the paralysis method was further used in a study of motor paralysis. In the first series of tests the reactions of *Phormia* flies 2 to 6 days old were compared with those of *Phormia* flies 23 to 30 days old. The first-reaction time and the revival time of the young flies did not differ greatly among themselves, but they did differ widely from those of the old flies. All the injected flies were totally paralyzed, but the period of paralyzation depended on the percentage of nicotine used and the age of the flies. The old flies were the more easily paralyzed and the paralysis lasted longer.

Tests were also made with six sets of *Phormia* flies ranging in age from 9 to 16 days. Flies of the age indicated were injected with doses of 1.24 mm^3 of nicotine solution of the following concentrations: 0.031 (9 days), 0.062 (10 days), 0.125 (11 days), 0.25 (12 days), 0.5 (15 days), and 1 percent (16 days). The weights of pure nicotine in these doses were 0.0004, 0.0008, 0.0016, 0.0031, 0.0062, and 0.0124

mg. The curves for first-reaction time (*a*) and revival time (*b*) in figure 4 start near 0 minutes when the smallest dose was used, and gradually ascend as practically straight lines. The curve for the first-reaction time is continued the full distance, but that for the revival time runs only one-fourth of the distance, because most of the flies failed to revive when they were given doses of 0.0062 and 0.0124 mg. Increased age caused these lines to ascend slightly, but increased dose caused most of the rise, as shown in previous tests.

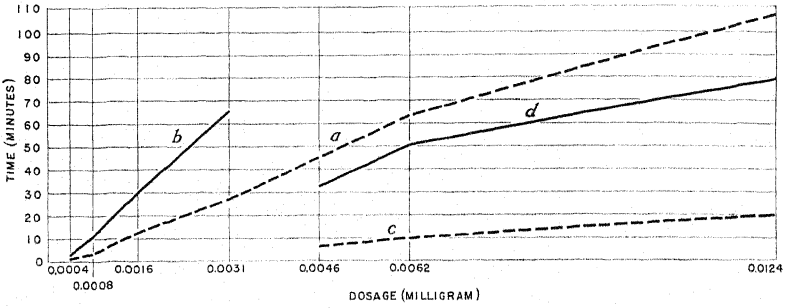


FIGURE 4.—Effect of increasing doses of nicotine on first-reaction time and revival time: *a*, First-reaction time, and *b*, revival time, of *Phormia*; *c*, first-reaction time, and *d*, revival time, of *Calliphora*

Three sets of *Calliphora*, 7, 8, and 10 days old, were injected, respectively, with 2.48 mm³ of 0.125-, 0.25-, and 0.5-percent nicotine solutions. Since this dose of the 0.125-percent solution paralyzed only half the females, a 3.72-mm³ dose was then used. The weights of pure nicotine in this dose were 0.0046, 0.0062, and 0.0124 mg. Again the curves (fig. 4, *c* and *d*) gradually ascend as practically straight lines; and since the three sets of flies were of nearly the same age, increasing age had practically no influence on these curves. Table 6 shows that the males were always more susceptible to nicotine than the females. The susceptibility ratio in this case was obtained by dividing the weight of the flies by the weight of nicotine injected, multiplying this quotient by revival time, and dividing this product by 1,000.

TABLE 6.—*Susceptibility of Calliphora flies to nicotine as shown by a study of motor paralysis*

Nicotine injected (mg)	Sex of flies	Weight of flies	First-reaction time	Revival time	Ratio of susceptibility
		Mg	*Minutes*	*Minutes*	
0.0046	Females	60	1.4	10.4	136
	Males	50	11.2	55.7	605
	Average	----------	6.3	33.0	----------
.0062	Females	60	9.6	47.0	455
	Males	50	10 9	64.8	522
	Average	----------	10.2	55.9	----------
.0124	Females	67	13.6	57.7	312
	Males	60	24.1	100.0	484
	Average	----------	18.9	78.8	----------

SYMPTOMS OF NICOTINE POISONING

In 1916 the writer (*3*) gave the first detailed description of nicotine poisoning in insects (bees). The information given here supplements that description.

When flies (*Phormia*) were totally paralyzed, the following symptoms were observed: About a second after the injection, while a fly was still in the glass holder, the abdomen, legs, and wings ceased moving vigorously, but quivered. The legs were usually folded together and the wings were bent toward the body. When a fly was quickly transferred to its vial, it often buzzed around on its back and kicked its legs, but the vigorous movements soon ceased, and then the wings were usually drawn to the body and the legs were folded together. The proboscis was always extended for a short time, then usually retracted, but it was common for a fly to die with its proboscis extended. A droplet of liquid often appeared on the proboscis and at the anus.

The following records pertain to injected larvae of various species: About 5 seconds after the tent caterpillars had been injected, convulsions occurred near the wounds and lasted half a minute. Later the larvae were completely paralyzed. When distilled water was used as a control, there were no convulsions and no signs of paralysis. Many of the injected silkworms spewed at the mouth and voided feces and liquid at the anus. Only slight movements were seen in the injected segments, but the integument often turned brownish or even darker. Movements of the same kind were also seen in the injected segments of southern armyworms. Codling moth larvae did not exhibit convulsions but only wriggled feebly. *Phormia* larvae showed only weak muscular movements near the needle, which was inserted at some distance from the visible heart. The nicotine did not seem to affect the heartbeat.

EFFECT OF POINT OF INJECTION ON TOXICITY

Hockenyos and Lilly (*1*) found that the toxicity of nicotine increased as the point of injection approached the head, where the largest ganglia occur. To ascertain whether the same is true in regard to the abdominal ganglia, three groups of 10 *Phormia* females, 13 days old, were injected between the second and third abdominal segments with 0.25-percent nicotine solution, the dose being 1.24 mm^3. Flies of the first group were injected on the ventral midline, those of the second on the dorsal midline, and those of the third, as usual, on the left side midway between these two lines. In the first group the needle went into or near the abdominal ganglion, in the second group it went into or near the heart, but in the third group it did not directly injure either of these vital organs. These tests were repeated with 30 *Phormia* males 9 days old. From the data in table 7 it is clear that the nearer the needle approached the ventral ganglion the more effective was the nicotine.

The foregoing tests were repeated by injecting 10 *Phormia* females in the mesothorax on the ventral midline. The reaction times were slightly less than those given in table 7 for females injected in the

abdomen on the ventral midline. Distilled water, used as a control, caused no signs of paralysis.

Other flies, which had been injected on the left side the previous day, were injected a second time in the wounds, which were well healed. This time the reaction times were slightly increased.

TABLE 7.—*Effect of point of injection on toxicity of nicotine to Phormia flies, as determined by the paralysis method*

Point of injection	First-reaction time			Revival time		
	Females	Males	Average	Females	Males	Average
	Minutes	*Minutes*	*Minutes*	*Minutes*	*Minutes*	*Minutes*
Dorsal midline	20.0	14.7	17.3	64.8	40.8	52.8
Left side	23.5	24.7	24.1	87.7	70.8	79.2
Ventral midline	31.0	32.6	31.8	114.2	118.7	116.4

SUMMARY

To inject minimum quantitative doses of nicotine into insects, a micrometer syringe and other devices to accompany it were used for the first time for administering insecticides. This apparatus was extremely accurate. With a minimum dose of 1.24 mm^3 the error never exceeded 4 percent and was usually less.

The comparative susceptibility to nicotine of various species and the sexes has been found to be of the following descending order: *Lucilia* male, *Lucilia* female, codling moth larva, *Calliphora* male and female, silkworm, armyworm, and *Phormia* larva. The *Lucilia* male flies were nine times as susceptible as the *Phormia* larvae. In general, the susceptibility of the larval forms was about half that of the imaginal forms.

The age of the injected flies greatly affected their susceptibility to nicotine. From the first to the fourth or fifth day susceptibility rapidly decreased; thereafter it less rapidly increased. Flies 14 days old were eight times as susceptible as flies 5 days old. The *Phormia* male flies were always more susceptible than the *Phormia* females of the same age.

It was shown statistically that solutions of free nicotine were from 22 to 26 percent more toxic than solutions of nicotine sulphate containing the same percentage of nicotine. However, when the nicotine in the nicotine sulphate solution was freed by the use of potassium hydroxide, its toxicity was equal to that of the other free nicotine.

In a study of motor paralysis old *Phormia* flies were more easily paralyzed than young flies and the paralysis lasted longer. For flies differing little in age the reaction times to increasing doses of nicotine can be represented by ascending straight lines. Males of *Calliphora* were more susceptible than females.

The first symptom of nicotine poisoning was stimulation, and this was followed by depression.

The toxicity increased as the point of injection approached the ventral ganglion.

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RESISTANCE OF SHEEP OF DIFFERENT BREEDS TO INFESTATION BY OSTERTAGIA CIRCUMCINCTA[1]

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INTRODUCTION

On August 29, 1935, 29 lambs, representing 5 breeds and 1 group of cross-breds, were separated from the general flock of sheep on the university farm at Davis, Calif., and used in an attempt to determine whether or not breed resistance to *Ostertagia circumcincta* (Stad.) existed in the breeds at hand. The breeds and birth dates of these animals are given in table 1.

TABLE 1.—*Breeds, brand numbers, and birth dates of lambs used to determine breed resistance to Ostertagia circumcincta*

Breed	Brand No.	Date of birth	Breed	Brand No.	Date of birth
Rambouillet	1	Dec. 1, 1934	Southdown	20	Jan. 31, 1935
	5	Dec. 10, 1934		19	Feb. 16, 1935
	3	Dec. 22, 1934		17	Mar. 7, 1935
	2	Dec. 24, 1934		18	Mar. 12, 1935
	4	Dec. 28, 1934		21	Mar. 16, 1935
Hampshire	26	Jan. 8, 1935	Romney	8	Feb. 7, 1935
	22	Feb. 21, 1935		9	Feb. 22, 1935
	25	Mar. 6, 1935		6	Feb. 24, 1935
	24	Mar. 9, 1935		10	Mar. 2, 1935
	23	Mar. 19, 1935		7	Mar. 15, 1935
Shropshire	12	Dec. 30, 1934	Cross-bred	27	Mar. 19, 1935
	16	Feb. 13, 1935	Shropshire-Merino	28	Mar. 20, 1935
	11	Mar. 1, 1935	Romney-Shropshire	29	Mar. 15, 1935
	13	Mar. 2, 1935			
	15	Mar. 15, 1935			
	14	Mar. 17, 1935			

Up to the beginning of the experiment these lambs were raised in the manner customary at the university farm. At the age of 2 weeks all were docked and the males were castrated. They were then pastured on native grass until about May 1, 1935, at which time they were turned into a field of Ladino clover. This field was divided into two pastures and was irrigated every 10 or 12 days. During irrigation the sheep were moved from one pasture to the other. Throughout the entire period the animals were brought to the barn every morning and fed hay and grain. They did not thrive well, however, and there was considerable scouring, which was first noticed about June 1.

All the lambs were weaned by June 15 and they were continued on Ladino clover with a grain ration once a day. On July 5 a diagnosis of coccidiosis was made in several lambs and the entire flock was treated daily for 5 days with bismuth and tannic acid in mineral oil

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[2] The authors desire to express their deep appreciation to Dr. Paul W. Gregory for advice and assistance in connection with the statistical analyses, to Dr. E. B. Roessler for checking the mathematical procedure, and to Dr. George H. Hart for criticisms and suggestions.

and fed exclusively upon dry hay. Some improvement was noted, but on July 18 a sick lamb was autopsied and a considerable infestation of *Ostertagia circumcincta* was discovered. The entire flock was treated on July 22, and again on August 5, with 2 ounces of a 1-percent solution of copper sulphate. The animals were fed hay and some grain and allowed to graze to a limited extent on Ladino clover from July 22 to August 29, when the experimental work began, and they manifested a gradually improved condition.

On August 29 all of the selected lambs with the exception of the Hampshires, were returned to Ladino clover pastures and kept there exclusively throughout the experiment. The Hampshire lambs were placed on this pasture September 6. Irrigation every 10 or 12 days was discontinued during the winter season when the pastures remained naturally moist. By September 20 the lambs appeared to be more thrifty and in better condition than for several months.

METHOD OF PROCEDURE

A fecal sample was obtained from each animal, and each lamb was weighed regularly every 2 weeks.

Fecal samples were collected by manual manipulation and the hands were thoroughly washed after each sampling to prevent contamination of succeeding samples. Each sample was wrapped in a piece of glazed onionskin paper bearing a number corresponding to the eartag. The samples were then taken to the laboratory and egg counts were made within 24 hours after the samples were collected.

The technique employed in examining the feces for eggs was a slight modification of Caldwell's method and was as follows:

(1) 1 g of feces was weighed out and placed in the bottom of a 30-ml shell vial calibrated at 10 ml.

(2) 2 ml of 30-percent antiformin was added to the feces in the shell vial and mixed thoroughly by means of a clean glass rod. The glass rod was left in the vial and the sample was allowed to stand at room temperature for at least 1 hour.

(3) Sugar solution with a specific gravity of 1.23 was added to the 10-ml mark on the vial.

(4) The glass rod was removed after stirring and the specimen was "bubbled" by means of blowing through a pipette placed against the bottom of the vial.

(5) 0.1 ml of the prepared specimen was taken from the vial with a pipette and spread on a slide so as to form a thin rectangular film.

(6) The eggs were counted with the aid of a mechanical stage under the 32-mm objective of the microscope. The number of eggs found in each smear was multiplied by 100 to express the number of eggs per gram of feces.

This slight modification of Caldwell's method was made to enable the writers to deal with smaller quantities of fecal material. Two slides of each specimen were prepared and counted for ova and the results were averaged to reduce error. The physical consistency of each specimen at the time of weighing in the laboratory was taken into consideration. A relationship of 1:2:4 is considered to exist between the formed, mushy (unformed), and diarrheic feces.

EXPERIMENTAL DATA

Figure 1 shows the average infestation, based upon the number of eggs per gram of feces, of each of the six groups of lambs and the average for the composite band from September 6, 1935, to September 4,

1936. Since the entire band was kept under identical conditions and handled in exactly the same way throughout the investigation, the egg count may be considered as a measure of resistance or susceptibility, and it is commonly accepted as such regardless of the true nature of the resistance. Assuming, then, that the egg count indicates the degree of infestation in each group, figure 1 shows an ascending order of susceptibility as follows: Romney, cross-breds, Southdown, Rambouillet, Shropshire, and Hampshire. Details of this variation in resistance or susceptibility, following exactly the egg counts made at intervals of 2 weeks, are shown in figure 2. The arrangement of the curves for the various breeds is such that in any given section of the abscissa the egg count shown for each breed was not only obtained from animals maintained under identical climatic and food conditions

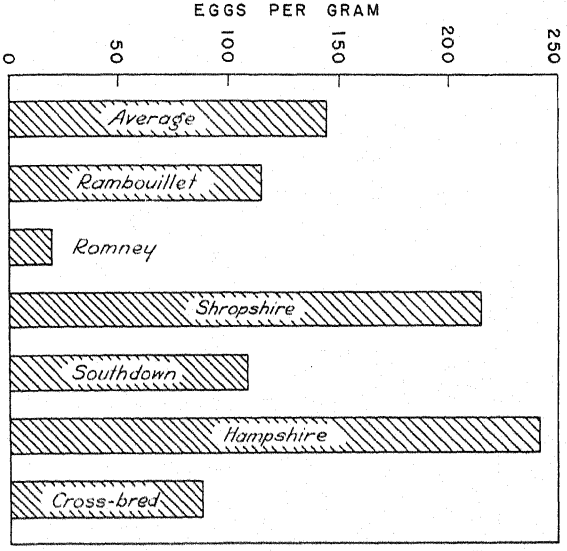


FIGURE 1.—Average infestation of *Ostertagia circumcincta* in the breeds of sheep investigated, and the average infestation for the entire band.

but from animals of practically the same age. Thus there is eliminated any possible confusion consequent upon making a comparison based upon age immunity or acquired immunity rather than upon natural resistance irrespective of age or degree of exposure to infection. It will be observed that figure 2 is in agreement with figure 1 with such minor exceptions as might be expected from a comparison of detailed data with averaged data. The peak attained by the Hampshires at 410 days was due very largely to an unusually heavy infestation in a single animal.

In order to determine whether or not such differences in resistance were significant, especially in view of the fact that a small number of each breed was under investigation, the data were analyzed by means of Fisher's pairing method.[3] The results are shown in table 2.

[3] FISHER, R. A. STATISTICAL METHODS FOR RESEARCH WORKERS. Ed. 4, 307 pp., illus. Edinburgh and London. 1932. See pp. 111–114.

TABLE 2.—*Results of analyzing the data regarding infestation by means of Fisher's pairing method in order to make comparisons between breeds*

Breeds compared	Sheep and mean egg count per gram of feces	*t* value[1]
Rambouillet with Southdown	Rambouillet 115, Southdown, 109	2.0109
Rambouillet with Hampshire	Hampshire 242, Rambouillet, 115	2.8891
Rambouillet with cross-bred	Rambouillet 115, cross-bred, 88	1.3614
Rambouillet with Romney	Rambouillet 115, Romney, 20	3.5854
Rambouillet with Shropshire	Shropshire 215, Rambouillet, 115	2.1824
Romney with Shropshire	Shropshire 215, Romney 20	4.3232
Romney with Southdown	Southdown 109, Romney 20	2.8008
Romney with Hampshire	Hampshire 242, Romney 20	3.5277
Romney with cross-bred	Cross-bred 88, Romney 20	2.9670
Shropshire with Southdown	Shropshire 215, Southdown 109	2.1200
Shropshire with Hampshire	Hampshire 242, Shropshire 215	.3783
Shropshire with cross-bred	Shropshire 215, cross-bred 88	2.6708
Southdown with Hampshire	Hampshire 242, Southdown 108	2.0577
Southdown with cross-bred	Southdown 109, cross-bred 88	.7171
Hampshire with cross-bred	Hampshire 242, cross-bred 88	3.0372

[1] The *t* value necessary to indicate significance is 2.479; high significance, 2.779.

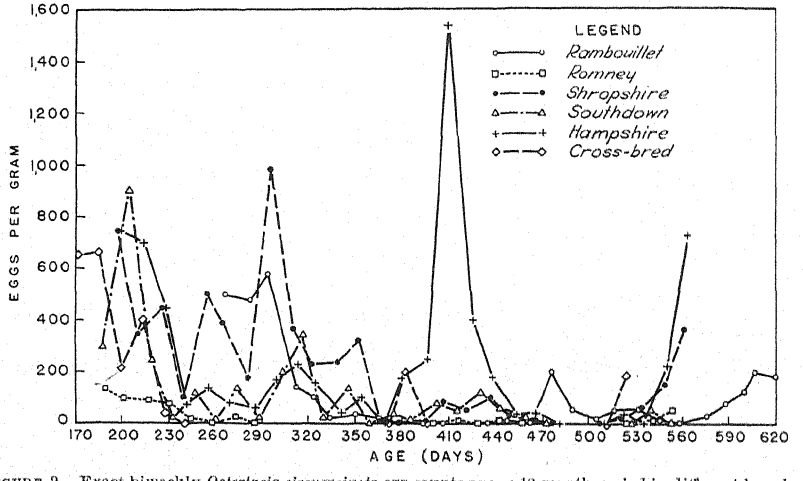


FIGURE 2.—Exact biweekly *Ostertagia circumcincta* egg counts over a 12-month period in different breeds of sheep.

It should be mentioned here that the calculations shown in table 2 are based upon the pairing of data obtained at each time of fecal sampling rather than upon mean egg counts for the entire period of investigation. It should also be pointed out that the *t* values, which are measurements of the significances of differences, where Southdowns and cross-breds are concerned are not based upon as many records as are the *t* values for other breeds, since out of five initial Southdowns, two died (one on March 11, 1936, and one on June 12, 1936), and out of only three initial cross-breds, one died on October 21, 1935. The cross-breds, however, may well be ignored since they do not represent a typical breed and are not considered further in this paper.

Extending the statistical analyses to include a study of possible significant differences in resistance between individual members of a single breed as well as differences between breeds, the results shown in table 3 were obtained.

TABLE 3.—*Results of analyzing the data regarding infestation by means of Fisher's pairing method in order to make comparisons between individuals*

Individual sheep compared	*t* value	*t* value necessary to be significant	*t* value necessary to be highly significant	Individual sheep compared	*t* value	*t* value necessary to be significant	*t* value necessary to be highly significant
Rambouillet 2 with 1	2.125	2.508	2.819	Shropshire 13 with 15	.359	2.518	2.831
Rambouillet 3 with 1	4.119	2.485	2.787	Shropshire 13 with 16	2.018	2.508	2.819
Rambouillet 4 with 1	1.795	2.492	2.797	Shropshire 14 with 15	1.458	2.583	2.921
Rambouillet 5 with 1	2.075	2.485	2.787	Shropshire 14 with 16	2.518	2.567	2.898
Rambouillet 3 with 2	1.851	2.500	2.807	Shropshire 15 with 16	3.312	2.539	2.861
Rambouillet 2 with 4	2.593	2.508	2.819	Southdown 17 with 18	.371	2.681	3.055
Rambouillett 5 with 2	.059	2.500	2.807	Southdown 17 with 19	3.164	2.528	2.845
Rambouillet 3 with 4	4.091	2.485	2.787	Southdown 17 with 20	1.741	2.624	2.977
Rambouillet 3 with 5	3.011	2.479	2.779	Southdown 17 with 21	2.759	2.539	2.861
Rambouillet 5 with 4	1.030	2.485	2.787	Southdown 18 with 19	1.403	2.681	3.055
Romney 8 with 7	.657	2.479	2.779	Southdown 18 with 20	.847	2.718	3.106
Romney 9 with 7	.148	2.479	2.779	Southdown 18 with 21	1.331	2.718	3.106
Romney 7 with 10	1.360	2.500	2.807	Southdown 20 with 19	3.181	2.583	2.921
Romney 8 with 9	1.426	2.508	2.819	Southdown 21 with 19	.989	2.508	2.819
Romney 8 with 10	2.088	2.528	2.845	Southdown 20 with 21	2.778	2.624	2.977
Romney 9 with 10	2.017	2.500	2.807	Hampshire 22 with 23	1.428	2.583	2.921
Shropshire 11 with 12	1.993	2.528	2.845	Hampshire 24 with 22	1.264	2.500	2.807
Shropshire 11 with 13	1.770	2.508	2.819	Hampshire 25 with 22	2.454	2.500	2.807
Shropshire 11 with 14	1.218	2.567	2.898	Hampshire 22 with 26	1.335	2.508	2.819
Shropshire 11 with 15	1.659	2.552	2.878	Hampshire 24 with 23	2.876	2.552	2.878
Shropshire 11 with 16	2.157	2.528	2.845	Hampshire 25 with 23	4.163	2.552	2.878
Shropshire 13 with 12	1.808	2.508	2.819	Hampshire 26 with 23	.979	2.567	2.898
Shropshire 14 with 12	2.465	2.539	2.861	Hampshire 24 with 25	.048	2.485	2.787
Shropshire 15 with 12	2.592	2.552	2.878	Hampshire 24 with 26	1.580	2.485	2.787
Shropshire 12 with 16	.702	2.528	2.845	Hampshire 25 with 26	4.263	2.492	2.797
Shropshire 14 with 13	.990	2.518	2.831				

In the Rambouillets three pairings showed highly significant, one pairing significant, and the remaining six pairings showed insignificant differences in susceptibility to *Ostertagia circumcincta*. The animal which showed a significant difference also showed a highly significant difference upon pairing with another animal. In the Romneys all pairings, six in number, showed no significant differences in susceptibility. In the Shropshires 2 pairings of a total of 15 showed significant differences in susceptibility—1 significant, the other highly significant. In the Southdowns a total of 10 pairings was made of which 2 were significant and 2 were highly significant; 1 animal being significantly resistant in 2 pairings and another being highly significantly resistant in 2. In the Hampshires one pairing was significant, two were highly significant, and the remaining seven were not significant. The animal showing a significant difference was also, in another pairing, one of those showing highly significant differences.

Having thus established not only a significant difference in susceptibility to *Ostertagia circumcincta* among different breeds but also among different individuals of the more susceptible breeds, the writers compared the more resistant individuals of each of the more susceptible breeds, again employing Fisher's pairing method, with the mean of the Romney breed. The results obtained are shown in table 4.

TABLE 4.—*Results of analyzing the data regarding infestation by means of Fisher's pairing method in order to make comparisons between the more resistant individuals of each of the more susceptible breeds with the mean of the Romney breed*

Breed and sheep no. compared with Romney	*t* value	*t* value necessary to be significant	*t* value necessary to be highly significant	Breed and sheep no. compared with Romney	*t* value	*t* value necessary to be significant	*t* value necessary to be highly significant
Rambouillet 1	0.965	2.485	2.787	Southdown 19	1.510	2.492	2.797
Rambouillet 4	1.792	2.485	2.787	Southdown 21	.263	2.500	2.807
Rambouillet 5	2.758	2.479	2.779	Hampshire 23	.919	2.552	2.878
Shropshire 12	2.194	2.508	2.819	Hampshire 26	2.454	2.485	2.787
Shropshire 16	1.158	2.500	2.807				

Of the three Rambouillets showing a significant degree of resistance only one showed a significant difference, which was nearly highly significant, from the Romney mean, indicating that two of the three animals possess a degree of resistance of approximately the same magnitude as that obtaining in the Romneys, the most resistant of the breeds studied. Of the two more resistant Shropshires both showed insignificant *t* values when paired with the Romneys. Of the two more resistant Southdowns both showed no significant difference when compared with the Romney mean. Of the two Hampshires showing significant and highly significant *t* values neither showed differences from the Romney mean which were significant.

CONCLUSIONS

From figure 1 it appears that the breeds of sheep used in this investigation vary in susceptibility to infection with *Ostertagia circumcincta* in the following ascending order: Romney, cross-bred, Southdown, Rambouillet, Shropshire, and Hampshire. The Romneys showed very many fewer eggs per gram of feces throughout a period of 12 months than the average for all the breeds, and an even greater difference is apparent when comparisons are made with the Shropshires and Hampshires. The cross-breds, Southdowns, and Rambouillets approximate one another in egg count, occupying a position about midway between the Romneys and the Shropshires and Hampshires, which approximate one another. The picture presented by this chart of average infestations is substantiated on the whole by figure 2, allowing for certain discrepancies which are to be expected when averages of data are compared with detailed data. Statistical analysis of detailed data, as presented in table 2, shows, however, that figure 1 does not give an entirely true picture. This analysis shows the Romneys to be highly significantly more resistant than any of the other breeds; the cross-breds, which may be ignored because they do not represent a true type, are significantly more resistant than the Shropshires and highly significantly more so than the Hampshires, but not significantly more resistant than any of the other breeds; the Rambouillets are highly significantly more resistant than the Hampshires but not significantly more resistant than any other breed; the Southdowns, Shropshires, and Hampshires show no significant differences in resistance or susceptibility from one another and are not significantly more resistant than any other breed. This informa-

tion forces the writers to conclude that the correct order of ascending susceptibility is as follows: Romney, cross-bred, Rambouillet, Southdown, Shropshire, and Hampshire, the last three breeds possessing a susceptibility of approximately the same magnitude. Figure 2 and unpublished results of investigations of seasonal incidence of *Ostertagia circumcincta* infestations in these and other animals on irrigated pastures indicate that age immunity or acquired immunity do not confuse this picture of breed resistance. One might reasonably expect Romneys to be more resistant to internal parasites favored by moist conditions than most if not all other breeds of sheep since they were developed in the Kent marshes in England and were held under relatively concentrated conditions. Natural selection and selective breeding, even though not scientifically conducted, would strongly tend to make such resistance characteristic of the breed.

Further statistical analyses demonstrated that certain individual animals of the susceptible, as well as the intermediate, breeds possess a resistance to *Ostertagia circumcincta* of approximately the same magnitude as the mean of that of the Romneys. It would appear, therefore, that the factor, or factors, which determine resistance have become fixed in the Romneys and that this same factor, or factors, is also in the other breeds although not fixed as in the resistant breed. Consequently it may be assumed that if a sheep breeder is desirous of securing a breed resistant to this stomach worm he may do so by careful selection within any of the above-mentioned breeds, and probably within those not investigated as well, without the necessity of attempting to cross-breed with all of its attendant difficulties and with the possible sacrifice of other and desirable characteristics typical of certain of these breeds. A similar thing has been done by Ackert and his associates [4] at Kansas State College, where breed resistance in fowls to *Ascaridia* has been demonstrated and a strain of White Leghorns resistant to this parasite has been developed by the selection of the most resistant cockerels and pullets in the flock and carried through three generations by subsequent father-daughter and brother-sister matings.

The findings given herein demonstrate to be true Stoll's idea [5] that there probably exist strains and breeds of sheep which are resistant to helminths.

SUMMARY

A group of 29 lambs, representing 5 breeds, and 3 cross-bred individuals, was isolated and kept for 12 months on an irrigated pasture, where they were continuously exposed to infection from *Ostertagia circumcincta*. Egg counts, by a modification of Caldwell's method, were made regularly every 2 weeks throughout the period. Such counts were used as an indicator to determine relative resistance or susceptibility. The data thus obtained were statistically analyzed by Fisher's pairing method.

From these analyses it was shown that the different breeds vary in susceptibility to the parasite in the following ascending order: Romney, Rambouillet, Southdown, Shropshire, and Hampshire, the last three

[4] ACKERT, J. E. PARASITES OF POULTRY. Jour. Amer. Vet. Med. Assoc. (n. s. 43) 90: 351-352. 1937.
[5] STOLL, N. R. CERTAIN NET EFFECTS IN HELMINTHIC PARASITISM, WITH SPECIAL REFERENCE TO THE SHEEP HOST. Cornell Vet. 26: 171-179. 1936.

breeds possessing a susceptibility of approximately the same magnitude. The Romneys are outstandingly resistant.

Further statistical analysis showed that in each of the more susceptible breeds there are significant differences in susceptibility, but in the Romneys there are no significant differences between individuals. Still further analysis demonstrated that the great majority of the more resistant individuals of the more susceptible breeds possess a resistance of approximately the same magnitude as the mean of that of the Romney. Since this is true it is theoretically possible for a breeder to develop a resistant strain within any of the more susceptible breeds investigated without running the risk of sacrificing a characteristic of some particular breed which he wants to maintain.

GROWTH SUBSTANCES IN RELATION TO THE MECHANISM OF THE ACTION OF RADIATION ON PLANTS [1]

By H. W. POPP, *professor of botany*, and H. R. C. MCILVAINE, *research assistant*, *Pennsylvania Agricultural Experiment Station*

INTRODUCTION

That radiation exerts a strong formative influence on plants has long been recognized. The blue-violet end of the spectrum is particularly effective in this respect, as has been shown by the work of Schanz (*7*),[2] Popp (*4*), Shirley (*8*), and others (*5*). With most plants, this part of the spectrum causes decreased stature as compared with the red end. In all the work with ultraviolet radiation carried out by the senior author since 1921 and in many of the researches of other investigators, no effect of such radiation, except perhaps the destructive action of the extremely short wave lengths, has been more consistently found than the decreased stature of plants (*6*). An exposure of as little as 2 minutes per day, to the unscreened radiation from a quartz mercury-vapor lamp at a distance of 50 cm exerts a marked stunting effect on seedlings otherwise kept in diffused daylight or in darkness.

No satisfactory explanation of the mechanism of this action of radiation has as yet been advanced. Previous attempts in the writers' laboratories to explain it on the basis of changes in such chemical substances as occur in sufficient quantities to be analyzed chemically have so far proved unsuccessful, although investigations in this field have hardly attained a good start as yet. Hare and Kersten (*3*), as a result of their discovery that ultraviolet radiation destroys indole-3-n-propionic acid, have recently suggested that "a possible action of ultraviolet light upon the tryptophan within the plant may in some part explain the effect of ultraviolet light upon plants." This inference warrants further inquiry, since it has been shown that ultraviolet radiation destroys tryptophan in vitro, and that disagreeable odors such as might result from the breaking down of tryptophan and other indole derivatives have sometimes been observed when seedlings were exposed to distinctly injurious doses of ultraviolet. The recent interest in plant-growth substances or hormones, the rapidly accumulating experimental data on their characteristics and effects, and particularly the technique developed (*1*, *9*, *10*) for their quantitative determination, have furnished another means of approach to this problem.

The present investigation was undertaken for the purpose of determining whether the effects on the plant of short-wave radiation could be explained on the basis of the effect of such radiation on plant-growth substances. The work was begun in January 1936, as a part of a more extended investigation of the effects of radiation on plants which has been under way for several years. After about 3,000 tests

[1] Received for publication July 30, 1937; issued February 1938. Contribution No. 101, Department of Botany, The Pennsylvania State College. Paper No. 780 in the Journal Series of the Pennsylvania Agricultural Experiment Station. This work was supported by the radiation committee of the Division of Biology and Agriculture, National Research Council.

[2] Reference is made by number (italic) to Literature Cited, p. 936.

had been completed, a paper appeared by Burkholder and Johnson (*2*) reporting a somewhat similar investigation.

In general, these men found that intact seedlings or excised tips of seedlings of *Avena* and *Zea* exposed to the radiation from a quartz mercury-vapor lamp transmitted through 5 cm of distilled water in a glass cell or through a Corning No. 986 red-purple Corex A filter of 7 mm thickness, contained less growth substance, as determined by a modified Went method, than did controls kept in darkness, the reduction in growth substance being greater with increased exposure. That the effect of the radiation was not brought about by a downward diffusion of the growth substance away from the direction of the radiation was indicated by the fact that lower sections of treated seedlings showed no commensurate increase in growth substance. Further evidence that the effect of the radiation was a direct one was the fact that irradiated blocks of agar into which growth substance had previously been allowed to diffuse from excised coleoptile tips caused less curvature in decapitated *Avena* test plants than did control blocks not irradiated.

Ultraviolet radiation alone, as transmitted through Corning No. 986 red-purple Corex A glass, caused a marked reduction in growth substance as compared with the amount in controls. The test plants were exposed to radiation through this glass for 60 and 120 minutes at an intensity of 1,888 ergs/cm^2/sec., an exposure probably great enough to cause injury to the seedlings, since this glass transmits ultraviolet radiation down to 250 mμ. Burkholder and Johnson do not indicate whether injury occurred, but the writers have never been able to irradiate plants through this glass under the conditions stated, without injuring the exposed tissues. On the other hand, tests with the blue mercury line, 4,358Å, at an intensity of 278 ergs/cm^2/sec., indicated to Burkholder and Johnson "only small and perhaps insignificant differences in growth substance as compared with the darkened controls." It is likely that in these tests the intensity of the radiation was too low and the exposure time not great enough, for, if the stunting effect of such radiation as compared with darkness is to be attributed to reduction in growth substances, we should expect a significant difference between irradiated plants and controls. In these tests, 8 seedlings were illuminated for 55 minutes, 10 for 180 minutes, and 10 for 300 minutes. The average curvatures produced by these seedlings were 11.0°, 9.5°, and 8.7°, respectively, as against 10.9° for the controls in darkness. While there was probably too small a number of seedlings used under any condition to warrant reliable inferences, these results do at least indicate a consistent decrease in growth substance with length of exposure.

When excised tips of coleoptiles were subjected to unilateral illumination, the illuminated side contained less growth substance than the shaded side, but, strangely enough, when intact coleoptiles were thus treated, although they bent toward the light, the illuminated side contained more growth substance than the darkened side. No explanation is offered for these contradictory results.

While the Burkholder and Johnson paper was probably only intended as a preliminary one, since few tests were made under any one set of conditions, the results do indicate that the effect of radiation of short wavelengths is to inactivate growth substance. If this is to be used in explanation of the mechanism of the action of radiation

upon plant growth, it is necessary that the conditions of radiation that produce definite formative effects be the ones that are used in the studies of growth substances. With ultraviolet radiation, for example, the intensity, spectral range, and duration of exposure should be such as to cause the ordinary stunting of growth without seriously injuring the seedlings. In the present investigation an attempt was made to satisfy these conditions.

EXPERIMENTAL PROCEDURE

The test plants used in this investigation were turnips (*Brassica rapa* L.), variety Purple Top White Globe. Individually selected seeds were germinated on cotton and filter paper in sterilized Petri dishes, 50 seeds to a culture, and kept in darkness at 25° C. except during the radiation treatments. The seeds were evenly spaced in the germinators.

The source of radiation was a mercury-vapor lamp in quartz operated on a 110-volt alternating current circuit with rectifier. The intensity of radiation from this lamp at a distance of 50 cm, without any screen, was 21.65 watts (10^{-5}/cm^2). Three types of Corning glass filters were used: Noviol 0, transmitting down to 389 mμ; G586A, transmitting the region 300–436 mμ; and red-purple Corex A, transmitting the region 250–415 mμ. The total energy transmission of these filters was measured by means of an Eppley pyrheliometer of the Kimball and Hobbs type [3] and the distances from the plants to the mercury-vapor lamp were so adjusted as to equalize the intensities. In some cases, however, the plants were exposed at shorter distances. The lengths of exposures through screens transmitting ultraviolet radiation or to the unscreened arc were such as to minimize injury but to produce stunting effects on the seedlings. These periods of time were determined by the results of several years' work with such radiation. Seedlings irradiated through Noviol 0, which eliminates all ultraviolet, were given longer exposures.

The plants were usually exposed for the first time about 24 hours after being placed in the germinators, and daily or twice a day thereafter, usually for 3½ or 7 days. During irradiation a strong current of air was kept moving over the plants to minimize heating effects, although these were negligible because of the short periods of exposure. Immediately after irradiation the plants were returned to the dark chamber. Controls consisted of plants kept under all the conditions to which the test plants were exposed except the irradiation.

Tests for growth substances were made by a slight modification of the Went *Avena* method (*9*). The test plants used for this purpose were a pure-line selection of oats (*Avena sativa* L.) made by Dr. C. F. Noll of this station and chosen because of their purity and uniformity. In the first series (table 1) the tips (1 mm) of the turnip seedlings to be tested for growth substance were transferred to blocks of 3 percent agar, 2 by 2 by 1 mm, and the growth substance allowed to diffuse into them while they were kept for 2 hours in a moist chamber. These blocks were then cut in half, one half of each block used for the tests and the other half discarded. The halves used were transferred unilaterally to *Avena* coleoptiles that had been decapitated

[3] Loaned by the Eppley Laboratory, Inc.

2 hours previously and allowed to remain in a moist chamber. About 0.5 mm of the coleoptile tip was again removed before this transfer. *Avena* coleoptiles about 30 mm in length were chosen from seedlings grown in darkness for about 5 days. During all manipulations the *Avena* seedlings were fastened with special holders and glass tubes. After the transfer of the agar blocks to the decapitated coleoptiles, the latter were returned to the dark chamber for 2 hours, at the end of which time the angle of curvature of the coleoptiles was measured directly in a shadow box. All manipulations were carried out in photographically inactive light. In the later tests (table 2) the tips of the turnip seedlings were transferred directly to the decapitated coleoptiles and the angle of curvature measured. For comparison some of the *Avena* coleoptile tips were also transferred to agar and tested for growth substance. Likewise, the agar itself was tested. All results were treated statistically and probable errors determined.

TABLE 1.—*Mean curvatures obtained with agar blocks into which growth substance had diffused from tips of turnip seedlings*

[Seedlings irradiated over a period of 7 days and given a total of 7 exposures, 24 hours apart]

Screen	Daily exposure	Tests	Mean curvature
	Minutes	*Number*	*Degrees*
Unscreened arc	5	2,232	0.52±0.008
Noviol 0	20	240	2.80± .023
Control (total darkness)	0	1,808	3.10± .012

TABLE 2.—*Mean curvatures obtained by direct transfer of tips of turnip seedlings to decapitated Avena coleoptiles*

[Seedlings irradiated over a period of 3½ days and given a total of 7 exposures, 12 hours apart]

Screen	Semi-daily exposure	Tests	Mean curvature
	Minutes	*Number*	*Degrees*
Unscreened arc	5	672	0.03±0.005
Red-purple Corex A [1]	5	240	.10± .009
Red-purple Corex A [2]	5	144	.99± .013
G586A	40	624	.70± .012
G586A	20	504	1.70± .016
Noviol 0	20	576	4.90± .017
Control (total darkness)	0	1,983	5.40± .013

[1] 16 cm from lamp.
[2] 30 cm from lamp.

RESULTS AND DISCUSSION

In table 1 are given the results obtained by transferring agar blocks to the decapitated coleoptiles. The results shown in table 2 were obtained by direct transfers of the tips of the treated turnip seedlings to the decapitated coleoptiles. The latter method proved to be the better since the authors were more concerned with relative than with absolute amounts of growth substance. The growth substance present in the *Avena* coleoptile tips, as determined by the agar method, and in the agar itself is shown in table 3.

TABLE 3.—*Growth substance present in Avena coleoptile tips and in plain agar*

[Seedlings grown in dark; 3½ days old when tested]

Item	Tests	Mean curvature
	Number	*Degrees*
Avena tips	480	3.20 ±0.010
Plain agar	336	.0044± .002

An examination of tables 1 and 2 reveals that in every case irradiated plants produced lower curvatures and hence less growth substance than did the controls kept in total darkness. Furthermore, the shorter the wave lengths to which the plants were exposed, the lower were the curvatures. Thus the lowest curvatures were obtained with plants exposed to the unscreened arc, which transmitted down to about 235 mμ; the next lowest by the plants screened with red-purple Corex A, which transmitted down to 250 mμ, and the next by the plants screened with G586A, which transmitted down to about 300mμ. Increasing the length of exposure with the G586A screen or decreasing the distance with the red-purple Corex A screen still further reduced the curvatures. The plants screened with Noviol 0, which eliminates practically all ultraviolet but transmits the entire visible spectrum, gave less curvature than the controls, indicating that, although the presence of ultraviolet radiation was much more effective in reducing growth substance, this effect of radiation is not restricted to the ultraviolet region. This is in accord with what would be expected from the fact that the seedlings screened with Noviol 0 were distinctly shorter than the controls, though much longer than the plants exposed to ultraviolet radiation. The shortest plants were uniformly those exposed to the unscreened arc.

There was, therefore, a distinct correlation between the stunting effect of the radiation used and the degree of curvature produced in *Avena* coleoptiles. Since the number of tests conducted under each condition was very large, and the differences between test plants and controls were uniformly in the same direction, these results are significant. This is further shown by the statistical analysis. If the Went method of determining growth substance is accepted as accurate, these results indicate that radiation, and particularly the short wave lengths of the spectrum, causes a reduction in the amount of growth substance present in growing tips of exposed plants. If, furthermore, we accept the postulation of Went and others, that the elongation of stems is directly controlled by growth substance, these results indicate that radiation checks elongation of stems through its action on growth substances. In other words, this formative effect of radiation may be at least partly explained on the basis of inactivation of growth substance. The discovery by Burkholder and Johnson, previously referred to, that radiation inactivated growth substance which was allowed to diffuse into agar, lends further support to this conclusion.

SUMMARY

A study has been made of the amount of growth substance present in turnip seedlings kept in total darkness as compared with the amounts present in such seedlings exposed to the radiation from an unscreened mercury-vapor lamp or to this radiation as screened through Noviol 0, G586A, or red-purple Corex A glasses. Irradiated plants uniformly contained less growth substance, as determined by the Went method, than did controls in total darkness. The shorter the wave lengths to which the plants were exposed, the greater was the reduction in growth substance. Although ultraviolet radiation was more effective in reducing the amount of growth substance present in seedlings, plants exposed to only visible radiation likewise showed a lower amount than did the controls in darkness. Since the degree of stunting of the seedlings was definitely correlated with reduction in growth substance, and since growth substance has been shown to exert a controlling influence on stem elongation, these results are believed to support the thesis that the stunting effect of radiation upon plants may be at least partly attributed to the inactivation of growth substances.

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THE CARBON METABOLISM OF FUSARIUM LYCOPERSICI ON GLUCOSE[1]

By GORDON H. PRITHAM, *graduate scholar*, and ARTHUR K. ANDERSON, *professor of physiological chemistry, Pennsylvania Agricultural Experiment Station*

INTRODUCTION

This paper reports the results of the fourth in a series of studies begun by Anderson in 1924, when he published on the biochemistry of *Fusarium lini* (*1*).[2] The second and third papers were from this laboratory, one in 1933, the other in 1934 (*2*, *3*); both deal with *Fusarium oxysporum*. The present investigation was undertaken to obtain data on a third *Fusarium*, which would provide a basis of comparison with the previous studies.

REVIEW OF LITERATURE

One of the most important investigations of tomato wilt is that of White (*17*), who in 1927 made a study of 24 strains of *Fusarium lycopersici* with respect to pathogenicity, influence of the hydrogen-ion concentration of the medium on the growth of the organism, products of metabolism on a glucose medium, and the toxicity of these products to tomato plants.

In a study of the hydrogen-ion concentration of the soil as related to the *Fusarium* wilt of tomato seedlings, Sherwood (*14*) found that acid soils favor wilting, but that there is no limiting degree of acidity or of alkalinity at which the disease will not develop. May (*8*) found no correlation between pH or titratable acidity of five varieties of tomatoes and resistance to *Fusarium* wilt at any stage of growth. Scott (*13*) obtained maximum growth of *Fusarium lycopersici* at pH values between 4.5 and 5.3, the organism tending to change the pH of all solutions toward a constant value of 6.0.

In a recent study of the metabolism of *Fusarium lycopersici*, Luz (*7*) observed four phases in the changes in hydrogen-ion concentration of the medium brought about by the growth of the fungus. He ascribes these changes to the formation of organic acids and to the preferential absorption of ions by the fungus. He also found that, as soon as the glucose is exhausted, the organism very rapidly utilizes the ethyl alcohol which it produces.

A considerable amount of work has been done on the biochemistry of *Fusarium lini* by Anderson (*1*), Letcher and Willaman (*6*), White and Willaman (*15*, *16*), and Reynolds (*11*). This work, together with

[1] Received for publication Mar. 12, 1937; issued February 1938. Paper No. 791 in the Journal Series of the Pennsylvania Agricultural Experiment Station. This paper is part of a thesis presented by Gordon H. Pritham to the faculty of the Graduate School of The Pennsylvania State College, in partial fulfillment of the requirements for the degree of doctor of philosophy.

[2] Reference is made by number (italic) to Literature Cited, p. 948.

that of Birkinshaw, Charles, Raistrick, and Stoyle (*5*) on various species of *Fusarium*, is reviewed elsewhere (*3*). Reynolds (*10*, *12*) demonstrated in resistant flax plants a cyanogenetic glucoside, linamarin, which inhibited the growth of *F. lini*, and he postulated that the fungus may release enough hydrocyanic acid from a resistant plant to inhibit its own growth.

Anderson, Everitt, and Adams (*3*) repeated much of Anderson's earlier work, using *Fusarium oxysporum* instead of *F. lini*. They found that the metabolism of the two organisms on glucose is very similar, the principal difference being that *F. oxysporum* utilized very little of the ethyl alcohol which it produced, while *F. lini* used it to a marked degree. Anderson and Emmart (*2*) studied the effect of adding certain amino acids to the culture medium on the rate of carbon dioxide production by *F. oxysporum*. The amino acid studied had little effect on the metabolic rate.

GENERAL METHODS AND MATERIALS

Source of Fusarium lycopersici culture.—The culture of the organism used in this investigation was obtained from Dr. C. D. Sherbakoff, of the Tennessee Agricultural Experiment Station. It was isolated from Maryland tomatoes and was known as culture M. From this culture a monosporous culture was prepared by the authors. Transfers from this culture were used in the present study.

Preparation of the medium.—The medium used in this work had the following composition:

Ammonium nitrate	1. 00 g.
Magnesium sulphate	0. 25 g.
Monopotassium phosphate	0. 50 g.
Distilled water to make	1, 000 ml

To this stock solution, sufficient glucose was added to make an approximately 2-percent solution of glucose. The exact quantity of glucose in each lot of medium was determined by the Folin-Wu (*7*) method after autoclave sterilization.

Hydrogen-ion concentration.—The pH value of all solutions was determined electrometrically. The quinhydrone electrode was used in all cases except in the study of the influence of the hydrogen-ion concentration on the growth of the fungus, when the Bailey hydrogen electrode was employed.

Determination of combustible gases.—Since a low percentage recovery of carbon was obtained in preliminary work, where the methods were the same as those used in earlier work on *Fusarium lini* (*1*) and *F. oxysporum* (*3*), it was thought that possibly the fungus might produce some gases or volatile compounds which were not absorbed during aeration of the flasks for carbon dioxide. Consequently, a combustion tube, partially filled with ignited lead chromate and wire-form cupric oxide, as recommended by Pregl (*9*), and another barium hydroxide tower were added to the aeration train, the gases being passed through the hot combustion tube and into the second barium hydroxide tower after going through the first barium hydroxide tower. A significant amount of carbon was recovered by this method. That this was not merely some carbon dioxide which had escaped absorption in the first tower was shown by interposing another barium hydroxide

tower between the first one and the combustion tube. Very little carbon dioxide was obtained in this tower.

Determination of nitrogen in the mycelium.—Since Anderson and Schutte (*4*) have shown that, in the absence of chlorides, the residue from the determination of carbon by the wet combustion method can be used satisfactorily for the determination of nitrogen by the Kjeldahl method, the residue from each carbon determination after digestion was made strongly alkaline with saturated sodium hydroxide solution, the ammonia being distilled and titrated as in the usual Kjeldahl method.

The methods for the preparation and inoculation of the culture flasks, the determination of carbon dioxide, ethyl alcohol, residual glucose, organic acids, and carbon in the mycelium are essentially the same as those used in previous work (*1*, *3*).

THE REACTION OF THE MEDIUM IN RELATION TO GROWTH

OPTIMUM, MAXIMUM, AND MINIMUM pH

The technique used in studying the relation of the reaction of the medium to growth of *Fusarium lycopersici* was similar to that employed by Anderson (*1*) in his work with *F. lini*. The results are presented in figure 1. It is evident that *F. lycopersici* is very tolerant to extremes of acidity and alkalinity, the minimum pH value for growth being 1.88 and the maximum 11.06. These results correspond well with those obtained by White (*17*) with *F. lycopersici* and with those of Anderson (*1*) with *F. lini*. Luz (*7*), however, obtained no growth of *F. lycopersici* at pH values above 8.10. The lowest pH that he tried was 3.25, at which value the fungus grew well.

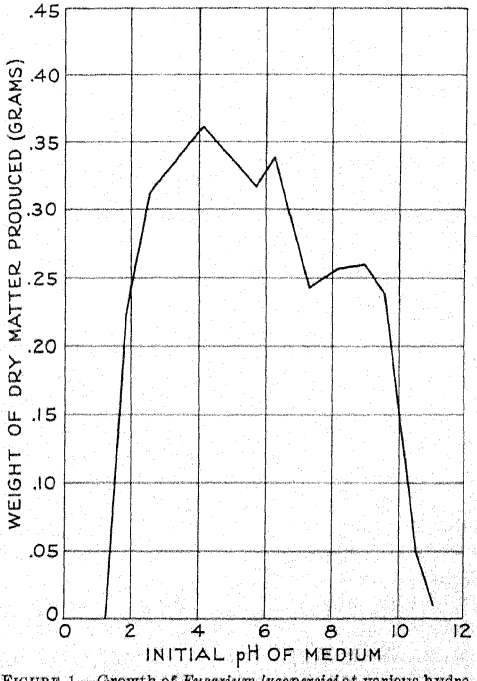


FIGURE 1.—Growth of *Fusarium lycopersici* at various hydrogen-ion concentrations.

The range for good growth is also very wide, extending from about pH 2.0 to pH 9.5, the optimum being pH 4.1. Scott (*13*) and White (*17*) obtained almost identical results with *F. lycopersici*, but observed two maxima in the growth curves at pH 4.0 to pH 5.3 and above pH 7, with a minimum between pH 5 and 7. Luz (*7*) observed maximum growth at pH 5.7. The results of the present study also agree well with those found for *F. lini* (*1*).

CHANGE IN pH DURING GROWTH

Figure 2 shows graphically the change in the reaction of the medium with time, both with and without the growth of the fungus. Perhaps the most striking effect is the marked tendency of the organism, within the range of vigorous growth, to change the reaction of the medium toward the optimum value. This effect is especially notable on the acid side, the final pH values of five cultures that had initial reactions of from 1.88 to 6.28 varying only from 4.25 to 4.95—values that are well within the optimum pH range. That these changes are due to the growth of the fungus and not to independent changes in the reaction of the medium itself is shown by the fact that the change in the reaction of the uninoculated medium tends to be constant. The

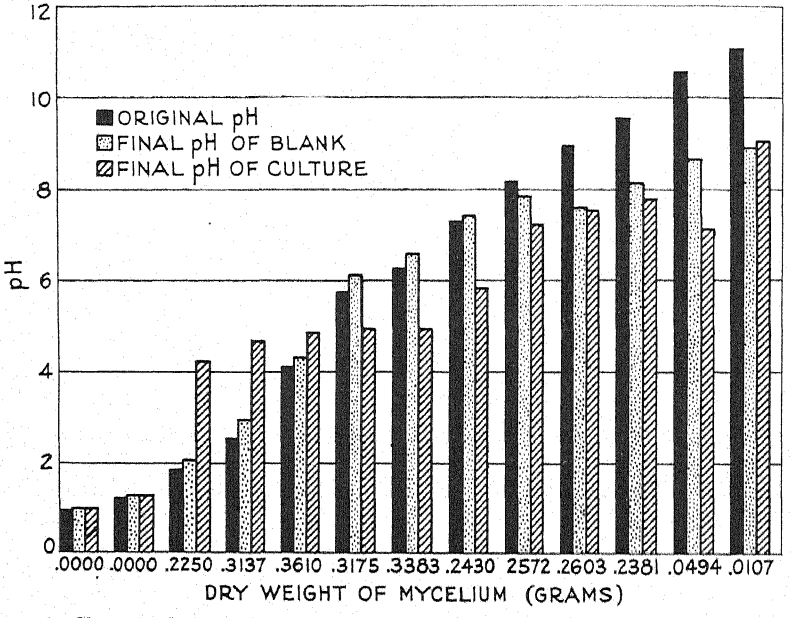


FIGURE 2.—Changes in hydrogen-ion concentration of the medium during growth of cultures shown in figure 1.

final pH of the blanks bears an approximately constant ratio to the initial pH, except in the strongly alkaline solutions, where there is evidently considerable absorption of carbon dioxide from the air. Although the change in the reaction of the inoculated medium is not so striking on the alkaline side as on the acid side of neutrality, the organism shows a decided tendency to increase the hydrogen-ion concentration toward the optimum. Part of this increase evidently is due to absorption of carbon dioxide from the air, but here again the change in the inoculated flasks is greater than in the uninoculated ones, indicating that the fungus itself changes the reaction of the medium. These results are similar to those obtained by Scott (*13*) with *Fusarium lycopersici*, and by Anderson (*1*) with *F. lini*.

In studies of the carbon metabolism on glucose, the change in pH was followed at intervals over a considerable period of growth. In all, four studies were made, and in every case the pH value of the medium was increased to about 7.0. There was no initial decrease, as Luz (7) observed in his work with *Fusarium lycopersici*, and as Anderson (1) found with *F. lini*. The pH value of an 8-months-old culture of *F. lycopersici*, which was used in testing for organic acids, was 7.3, showing that the fungus will change the reaction of the medium from acid to alkaline if allowed to grow long enough. Luz (7) ascribes the increase in pH to 7.5, as observed by him, to the preferential absorption of nitrate ions from ammonium nitrate in the medium, with the concurrent liberation of ammonium ions.

CARBON METABOLISM ON GLUCOSE

Although four studies (series) were made, the results of only the last will be presented in detail as it is representative of the other three (table 1 and fig. 3). Since in the first series the total recovery of the original carbon decreased to less than 50 percent as growth progressed, it was thought that the fungus might produce some compounds other than the ones being determined. Accordingly, two new methods were introduced in the subsequent series. It was suggested that some of the undetermined carbon might be in the form of gaseous or volatile compounds which would be lost during the aeration of the cultures for carbon dioxide. To test this hypothesis, a combustion tube and a barium hydroxide tower were introduced into the aeration train. By means of this technique, the results of which are given as "Combustion" in table 1 and figure 3, an amount of carbon representing about 2 percent of the original carbon was obtained, thus showing that a small amount of volatile organic compounds is produced by *Fusarium lycopersici*.

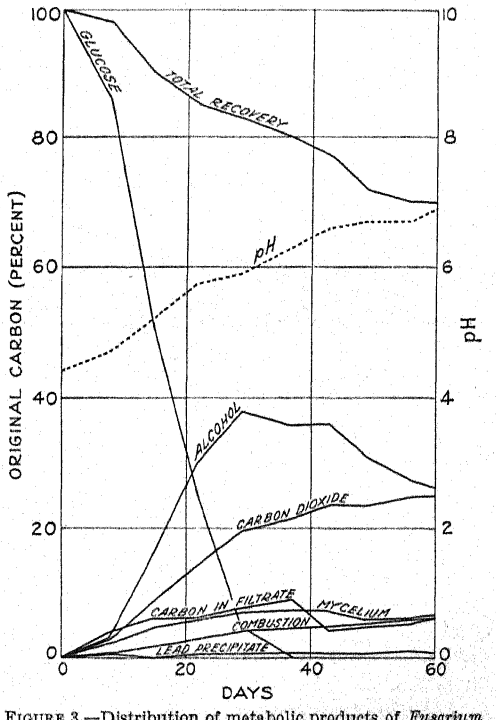


FIGURE 3.—Distribution of metabolic products of *Fusarium lycopersici* when grown on glucose. See table 1.

TABLE 1.—*Distribution of metabolic products of Fusarium lycopersici on a glucose medium at various stages of growth*

Age of culture (days)	Mycelium					Carbon dioxide		Ethyl alcohol	
	Weight	Weight of carbon	Carbon	Carbon as related to initial carbon [1]	Nitrogen	Weight of carbon	Carbon as related to initial carbon [1]	Carbon in 300 ml	Carbon as related to initial carbon [1]
	Gram	*Gram*	*Percent*	*Percent*	*Percent*	*Gram*	*Percent*	*Gram*	*Percent*
0									
8	0.120	0.081	45.88	2.31	3.62	0.068	2.97	0.075	3.20
15	.230	.110	47.79	4.70	3.22	.213	9.08	.374	15.96
22	.307	.140	45.62	5.98	3.33	.341	14.55	.712	30.44
29	.347	.169	48.56	7.20	4.27	.446	19.55	.890	38.01
37	.383	.177	46.26	7.58	4.03	.502	21.46	.825	35.76
43	.385	.176	45.91	7.54	4.00	.549	23.45	.841	35.96
49	.341	.143	41.63	6.12	3.68	.547	23.36	.706	30.73
56	.366	.147	40.00	6.26	3.38	.574	24.55	.644	27.32
60	.365	.156	42.67	6.65	3.62	.575	24.58	.612	26.11

Age of culture (days)	Glucose		Lead precipitate		Combustion		Residual carbon in filtrate		Total recovery of carbon as related to initial carbon [1]	pH
	Carbon in 300 ml	Carbon as related to initial carbon [1]	Weight of carbon	Carbon as related to initial carbon [1]	Weight of carbon	Carbon as related to initial carbon [1]	Weight of carbon	Carbon as related to initial carbon [1]		
	Gram	*Percent*	*Gram*	*Percent*	*Gram*	*Percent*	*Gram*	*Percent*	*Percent*	
0	2.34	100.0							100.0	4.4
8	2.01	85.8	0.010	0.42	0.024	0.70	0.06	4.00	98.0	4.7
15	1.19	50.9	.003	.12	.044	1.88	.15	6.20	90.0	5.2
22	.57	24.2	0.008	.40	.071	3.03	.15	6.20	85.0	5.8
29	.12	5.0	.020	.80	.101	4.30	.17	7.90	83.0	5.9
37	.01	.3	.023	1.00	.112	4.80	.20	8.70	80.0	6.3
43	.00	.0	.023	1.00	.119	5.07	.11	4.48	77.0	6.6
49	.00	.0	.021	.87	.128	5.35	.12	4.70	72.0	6.7
56	.00	.0	.028	1.15	.141	6.04	.13	5.30	70.0	6.7
60	.00	.0	.023	1.00	.143	6.10	.14	6.00	70.0	6.9

[1] Expressed as percentage of the carbon in the glucose originally present.

As a further check on the recovery of carbon, an aliquot of the filtrate from the original cultures was analyzed for total carbon by the wet combustion method. The sum of the carbon in the alcohol, residual glucose, and lead precipitate was then subtracted from this total carbon, giving the undetermined, or residual carbon in the filtrate. A maximum of 8.7 percent of the original carbon could be accounted for in this way, indicating that a significant amount of some organic compounds, other than ethyl alcohol, which are not precipitated by lead acetate, is formed by the organism from glucose. The curve for the total recovery of carbon in figure 3 includes both the residual carbon and the carbon obtained by combustion.

Table 1 and figure 3 show that ethyl alcohol and carbon dioxide are the main products of the metabolism of the organism on glucose, the alcohol being produced slowly at first and then more rapidly until the glucose has been exhausted, when it decreases fairly rapidly. Since carbon dioxide production contines to increase after the consumption of the glucose, it is evident that *Fusarium lycopersici* can utilize ethyl alcohol for energy and for growth. These facts are in accord with the results obtained by Anderson (*1*) with *F. lini*, except that the decrease in the alcohol, as well as the rate of metabolism for the entire series, is

not as rapid as he found it to be. Anderson, Everitt, and Adams (*3*) found that *F. oxysporum* was able to utilize ethyl alcohol as a source of carbon to only a slight extent. In this connection the recent work of Luz (*7*) should be mentioned. He found that *F. lycopersici*, when grown on a medium containing 5 percent of glucose, produced alcohol rapidly until the glucose was exhausted on the eighteenth day of growth, when the alcohol was used at a rapid rate until it was finally exhausted on the thirty-fifth day of growth. Since, in the present study, a considerable amount of alcohol was present in the medium after 60 days' growth, it is obvious that the rate of metabolism of the strain of *F. lycopersici* used by him was much greater than that of the strain employed in this investigation. However, his mineral nutrient solution was 10 times as concentrated as the one used in the present work.

The lead precipitate is significant because it is nearly constant, although representing only a small fraction of the original carbon. Evidently *Fusarium lycopersici* produces only small amounts of organic acids, the lead salts of which are insoluble. Anderson (*1*) also found that only small amounts of these acids were produced by *F. lini*.

The residual carbon in the filtrate increases rather rapidly until the glucose is practically exhausted, and then decreases. Since this decrease is at a somewhat more rapid rate than the corresponding decrease in alcohol and in the total carbon in the filtrate, it is evident that the fungus utilizes this carbon more easily than it does ethyl alcohol. As old cultures have an esterlike odor resembling that of apricots, it is possible that this residual carbon is represented by some organic acids which were not determined by any of the methods used.

The amount of volatile compounds determined by combustion shows a gradual increase during the entire growth period. The highest value was obtained in the last series at 60 days, when 6.1 percent of the original carbon was in this form. At least a part of this carbon may be in the form of volatile acids, since in subsequent experiments a small amount of these acids was found in old cultures of the organism.

The changes in the pH of the medium with growth are practically uniform in all series and the final pH is nearly the same in all series, being well within the optimum range for growth. Luz (*7*) found that he could divide the growth of *Fusarium lycopersici* into four stages on the basis of the pH changes during growth, but no such periods, or "breaks," in the pH curves were obtained in this work. Anderson (*1*) observed that *F. lini* first decreased the pH of the medium and then gradually increased it, the final value depending on the initial pH of the medium.

QUALITATIVE TESTS FOR ORGANIC ACIDS

The filtrate from an 8-months-old culture of *Fusarium lycopersici* was distilled from acid solution, and the distillate titrated with standard alkali. An amount of volatile acid equivalent to about 4.0 ml of normal acid, or 0.24 g of acetic acid, was obtained. The residue from the distillation was tested for oxalic acid by the usual precipitation as calcium oxalate. Since there was obtained a white precipitate which decolorized potassium permanganate in sulphuric acid solution, it was concluded that oxalic acid was formed in small amounts, thus confirming the findings of Luz (*7*). A doubtful test for tartaric acid was also obtained.

COMPOSITION OF MYCELIUM

The data for the percentage of carbon and of nitrogen in the mycelium, calculated on the dry weight of mycelium, are given in the column headed "Mycelium" in table 1. There is a tendency for the carbon content of the mycelium to decrease with age, a change exactly opposite to that found for *Fusarium lini* (*1*) and for *F. oxysporum* (*3*).

In general, the nitrogen in the mycelium tends to decrease slightly with age, indicating that old cultures contain less active protoplasm than fresh cultures. These results agree with those obtained for *Fusarium lini* (*1*).

Carbohydrates contain about 40 percent of carbon, proteins 51 to 55 percent, and a typical fat such as tristearin 76.87 percent. During the first 43 days of growth the carbon content of the mycelium ranged from 45.62 to 48.56 percent. During the final stages of growth the carbon content of the mycelium dropped to between 40.00 and 42.67 percent. Since the percentage of nitrogen in the mycelium decreased together with the percentage of carbon it appears that the carbohydrate content of the mycelium was increasing with age. There was no evidence of fat formation, as was the case with *Fusarium lini* (*1*) and *F. oxysporum* (*3*).

RATIO OF PRODUCTS OF METABOLISM TO ONE ANOTHER AND TO THE GLUCOSE CONSUMED

Various coefficients and equivalents have been introduced into the literature to express the relationships existing among the products of metabolism and the compounds consumed by a fungus. A number of these ratios have been calculated for each series of experiments in the present investigation; the ones for the last series are presented in table 2. To avoid confusion, the exact meaning of each ratio is given at the top of each column.

The respiration coefficient represents the grams of carbon dioxide produced per gram of dry mycelium. There is an increase in this ratio with age up to a certain point, and then a slight decrease, this point usually coinciding with the exhaustion of the glucose and the beginning of the utilization of ethyl alcohol. Evidently *Fusarium lycopersici* is able to use ethyl alcohol better for mycelium building than as a source of energy. *F. lini* (*1*) and *F. oxysporum* (*3*) showed a steady increase in this ratio.

TABLE 2.—*Quantitative relationships existing between the various metabolic products of Fusarium lycopersici when grown on a glucose medium*

Age of culture (days)	Respiration coefficient: $\frac{\text{Weight of } CO_2}{\text{Weight of mycelium}}$	Economic coefficient: Weight of sugar consumed / weight of mycelium	Respiration equivalent: Carbon of $CO_2 \times 100$ / carbon consumed	Plastic equivalent: Carbon of mycelium × 100 / carbon of glucose consumed	Alcohol equivalent: Carbon of alcohol × 100 / carbon of glucose consumed	Carbon of alcohol / Carbon of CO_2
8	2.08	6.83	20.60	24.72	22.70	1.10
15	3.39	12.46	18.50	9.57	32.49	1.76
22	4.07	14.42	19.25	7.92	40.20	2.09
29	4.72	16.02	20.10	7.59	40.70	2.00
37	4.83	15.23	21.56	7.59	35.40	1.64
43	5.22	15.19	23.46	7.54	35.95	1.53
49	5.88	17.15	23.37	6.11	30.15	1.29
56	5.76	16.00	24.52	6.22	27.48	1.12
60	5.77	16.04	24.60	6.66	26.17	1.07

Similar changes take place in the economic coefficient, which is the amount of carbon source required to make 1 g of dry mycelium, the ratio increasing to a maximum and then decreasing as the fungus changes from glucose to alcohol as a building material. *Fusarium lini* (*1*) showed similar changes, but the maximum value was only 13.1, as compared with 17.15 in the case of *F. lycopersici*. Evidently the former organism uses carbon sources more efficiently than the latter. That young mycelium is more efficient in sugar consumption than older mycelium is illustrated by the low value obtained in every case for the first growth period, usually a week or 10 days.

As would be expected, the percentage of the carbon consumed that is transformed into carbon dioxide, or the respiration equivalent, increases with age during the growth of the fungus, since ethyl alcohol is used as a source of energy after the glucose has disappeared.

The plastic equivalent is really the reciprocal of the economic coefficient, since it represents the percentage of the glucose consumed that is converted into mycelium. As would be expected, it shows reciprocal changes, decreasing with age until the glucose is entirely used, and then increasing slightly. These changes also tend to show that ethyl alcohol is used somewhat more efficiently than glucose for mycelium production.

The alcohol equivalent is the percentage of glucose consumed that is converted into ethyl alcohol. The changes in this ratio show that *Fusarium lycopersici* utilizes ethyl alcohol after it has used all the glucose, since the values increase until the point at which the glucose is exhausted is reached, after which they decrease markedly.

In the last column of table 2 the ratio of the carbon in alcohol to the carbon in carbon dioxide is given. In a typical alcoholic fermentation the ratio of carbon in alcohol to carbon in CO_2 is 2:1. Table 2 shows that the experimentally determined ratio approaches very closely the theoretical, and is exactly 2.00 on the day of maximum alcohol production. Because of the utilization of the alcohol by the fungus, the ratio decreases to 1.07:1 on the sixtieth day of growth, indicating that about 25 percent of the alcohol is changed into carbon dioxide. The actual decrease in alcohol is over 30 percent, the difference being accounted for by the growth of the mycelium.

UTILIZATION OF ETHYL ALCOHOL

Since *Fusarium lycopersici* utilizes the ethyl alcohol which it produces from glucose, the effect on the growth of the fungus of various concentrations of ethyl alcohol, both alone and in the presence of the same concentration of glucose that was used in the preceding work, was studied. Two series of 26 flasks each were used, each flask in one series containing 100 ml of mineral nutrient medium without glucose, and each flask in the other series containing the same amount of medium with enough glucose added to make an approximately 2-percent glucose solution. The exact amount of glucose was not determined, but was assumed to be the same in all flasks containing it, since the medium for each flask was measured from one large lot. To 24 flasks from each series, amounts of 95 percent ethyl alcohol varying from 0.2 to 6.0 ml were added after sterilization. Two flasks were used as controls, no alcohol being added. After 10

days growth, the mycelium was removed by filtration and dried to constant weight at 100° C. The results are presented in figure 4.

In low concentrations, ethyl alcohol is a good source of carbon for *Fusarium lycopersici*. The fungus will grow slightly, even in concentrations as high as 4.90 g per 100 ml of solution. That maximum growth occurred at a concentration of 0.41 g per 100 ml is significant, as the maximum amount of alcohol produced by the fungus in any of the studies of the glucose metabolism was 0.57 g per 100 ml. The limiting value for maximum growth is apparently between these two figures. These results compare favorably with those of Anderson (*1*), who obtained maximum growth of *F. lini* at an alcohol concentration of 0.38 g per 100 ml. A concentration of 3.59 g per 100 ml, however, inhibited completely the growth of this organism.

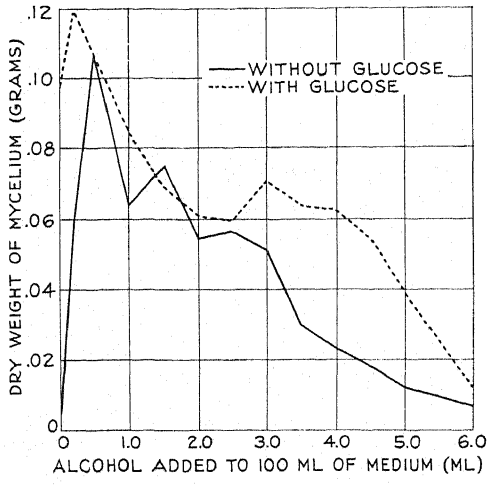


FIGURE 4.—Mycelium production on various concentrations of ethyl alcohol, with and without glucose.

As was expected, growth was generally heavier in the flasks containing glucose in addition to alcohol, although the two growth curves tend to parallel each other. That low concentrations of alcohol have a stimulating effect on the growth of the fungus on glucose is evidenced by the fact that the weight of mycelium produced on the solution containing 0.16 g of alcohol is significantly greater than that on the glucose solution alone. As the amount of alcohol increases, however, the weight of mycelium decreases until, at a concentration of 4.90 g per 100 ml, less mycelium is produced than on a solution containing 4.08 g of alcohol and no glucose.

DISCUSSION OF RESULTS

Fusarium lycopersici is as tolerant to extremes of hydrogen- and hydroxyl-ion concentration as *F. lini*, and the optimum pH value for growth is practically the same for both organisms. *F. lycopersici* is apparently better able to change the pH of strongly acidic solutions toward the optimum value than is *F. lini*. In view of the ability of the organism to grow over such a wide range of pH values, it would not appear feasible to attempt to control the *Fusarium* wilt of tomatoes by adjusting the pH of the soil, although Sherwood (*14*) found that there was a greater percentage of infection of tomato seedlings by the fungus in acid soils.

The results of the work on the carbon metabolism of *Fusarium lycopersici* on glucose indicate that this organism produces, in addition to ethyl alcohol and carbon dioxide, appreciable quantities of volatile and nonvolatile compounds, part of which was determined by new

methods. Because of the striking esterlike odor of old cultures, it is thought that fatty acids comprise an appreciable portion of these compounds, although attempts to identify these acids were unsuccessful.

The production of these compounds is an essential point of distinction between this organism and *Fusarium lini* and *F. oxysporum*. In the work with these last two fungi over 90 percent of the original carbon could be accounted for in the ethyl alcohol, carbon dioxide, and mycelium, whereas in the present study the maximum percentage of the original glucose that could be recovered in the form of these three products was less than 70 percent. This difference in the percentage recovery probably is due to actual differences in the biological characteristics of the organisms and not to faulty technique.

Another biochemical difference between the three species of *Fusaria* under discussion appears in the carbon content of old mycelia. In *Fusarium lycopersici* the carbon content of the mycelium approaches that of a carbohydrate, whereas with *F. lini* and *F. oxysporum* there is an indication of fat deposition with age.

The course of the alcoholic fermentation caused by *Fusarium lycopersici* is practically the same as that of *F. lini* and of *F. oxysporum*, alcohol and carbon dioxide being produced in approximately the same proportions by all three fungi. The amount of alcohol produced by *F. lycopersici*, however, was somewhat less than that produced by the other two organisms. The maximum quantity obtained in this work was 1.7 g, as compared with 2.1 to 2.2 g in the other investigations.

No attempts were made to discover the substances that are toxic to tomato plants. It does not seem likely, however, that the organism produces alcohol in sufficient quantity to account for its wilting effect, as the maximum concentration obtained in these studies was 0.57 g per 100 ml, and Luz (7) found that tomato plants were not wilted when placed in alcohol solutions containing 2 g per 100 ml. White (17) found that tomato cuttings were not wilted by concentrations of alcohol up to 4 percent by volume, but that the low molecular weight fatty acids and oxalic acid were highly toxic in concentrations of only 0.06 percent. Concentrations of alcohol of 4 percent or more inhibited the growth of the fungus, even in the presence of glucose, which is presumably an easily assimilable form of carbon; hence it is not likely that the concentration of alcohol in the tissues of the infected plant would become great enough to cause wilting and death of the plant.

SUMMARY

Fusarium lycopersici, the organism which causes tomato wilt, is not sensitive to extremes of hydrogen-ion and hydroxyl-ion concentration. It will grow on artificial media at pH values between 1.88 and 11.06.

The range for good growth is also wide, extending from pH 2.0 to pH 9.5.

The organism shows a decided tendency to change the pH of both acid and alkaline media toward the optimum value, the final pH in five cases varying only between pH 4.25 and pH 4.95.

The principal products of the metabolism of the organism on glucose are carbon dioxide and ethyl alcohol, but considerable quantities of other compounds, presumably volatile and nonvolatile organic acids,

are formed as well. Some of the carbon in these unknown compounds was determined by means of new procedures.

The ratio of the carbon in alcohol to carbon in carbon dioxide is very nearly that required by the equation for a typical yeast fermentation. Because of the consumption of the alcohol by the fungus during the later periods of growth, however, this ratio gradually decreases with the age of the culture.

Fusarium lycopersici definitely uses the ethyl alcohol that it produces for energy and growth as soon as the glucose in the solution has been exhausted. That it uses the alcohol somewhat more efficiently than the glucose is evidenced by the slow decrease in the plastic equivalent while the organism is using glucose and the sudden increase in this ratio when alcohol becomes the carbon source.

Ethyl alcohol will serve as the sole carbon source for *Fusarium lycopersici*, maximum growth being obtained on a concentration of alcohol approximating the maximum amount produced by the fungus on a glucose medium. Concentrations of alcohol of 4 percent by volume or more seriously inhibited the growth of the fungus. A small amount of alcohol (0.2 percent) stimulated mycelium production on a glucose medium, whereas concentrations of 5 percent by volume or more inhibited growth.

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